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June 26, 2000

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RE: New Patent Continuation-In-Part Application in U.S.
Applicant: Xue-Ru WU et al.
Title: TRANSGENIC ANIMALS AS URINARY BIOREACTORS FOR THE PRODUCTION OF POLYPEPTIDE IN THE URINE, RECOMBINANT DNA CONSTRUCT FOR KIDNEY-SPECIFIC EXPRESSION, AND METHOD OF USING SAME
Atty's Docket: WU=43C

Sir:

Attached herewith is the above-identified Continuation-In-Part application for Letters Patent including:

[X] Specification (59 pages), claims (7 pages) and abstract (1 page)
[X] 26 Sheets Drawings (Figures 1-20)
[X] Formal [ ] Informal
[X] The inventors of this application are:

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[ ] Information Disclosure Statement with ( ) references
[X] Return Receipt Postcard (in duplicate)

The following statements are applicable:

[ ] The benefit under 35 USC '119 is claimed of the filing date of: Application No. __ in ____ on _____. A certified copy of said priority document
[ ] is attached
[ ] was filed in progenitor case_____ on_____.
The present application claims the benefit of U.S. Provisional Appln. No. 60/ , filed.

[X] The present application is a [X] Continuation-in-part of prior application No. 09/438,785, which claims the benefit of U.S. Provisional Appln. Nos. 60/108,195, filed November 13, 1998 and 60/142,925, filed July 9, 1999. Although this application is stated to be a CIP, applicant does not concede that any matter is presented in this application which is not present in the parent.

[ ] Incorporation By Reference. The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied herewith, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

[ ] The undersigned attorney of record hereby appoints associate power of attorney, to prosecute this application and to transact all business in the Patent and Trademark Office in connection therewith to:

[X] Certain documents were previously cited or submitted to the Patent and Trademark Office in the following prior application 09/438,785, which is relied upon under 35 USC §120. Applicants identify these documents by attaching hereto a form PTO-1449 listing these documents, and request that they be considered and made of record in accordance with 37 CFR §1.98(d). Per Section 1.98(d), copies of these documents need not be filed in this application.

[X] In accordance with 37 CFR 1.53(a) and (b), it is respectfully requested that a serial number and filing date be assigned to this application as of the date of receipt of the present papers. In accordance with the present procedures of the U.S. Patent and Trademark Office, an executed Declaration and the filing fee for the present application will be filed in due course.

[X] **NO authorization is given for charging the filing fee at the present time. However, at such time that the declaration is filed, but not before, you are authorized to charge whatever excess fees are necessary (including the filing fee and any extension of time fees then due) to Deposit Account 02-4035, if any such fees due are not fully covered by check filed at that time.**

[X] The attorneys of record for this application and the address will be those of **Customer No. 001444**; i.e., Sheridan Neimark, Reg. No. 20,520; Roger L. Browdy, Reg. No. 25,618; Anne M. Kornbau, Reg. No. 25,884; Norman J. Latker, Reg. 19,963; Iver P. Cooper, Reg. No. 28,005; and *Allen C. Yun, Reg. No. 37,971 (*Patent Agent).

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Please direct all telephone calls to Browdy and Neimark at (202) 628-5197.

[X] The Commissioner is hereby authorized to credit any overpayment of fees accompanying this paper to Deposit Account No. 02-4035.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.

By: _____
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TRANSGENIC ANIMALS AS URINARY BIOREACTORS FOR THE PRODUCTION OF POLYPEPTIDE IN THE URINE, RECOMBINANT DNA CONSTRUCT FOR KIDNEY-SPECIFIC EXPRESSION, AND METHOD OF USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of application 09/438,785, filed November 12, 1999, which claims priority under 35 U.S.C. §119(e) from U.S. provisional application 60/108,195, filed November 13, 1998, and U.S. provisional application 60/142,925, filed July 9, 1999, the entire contents of each of these prior applications are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to transgenic animals as urinary bioreactors for the expression and production of polypeptides in the urine. The present invention further relates to a recombinant DNA construct for kidney-specific expression of polypeptides in the urine and to a method for producing such polypeptides in the urine.

Description of the Related Art

Significant progress has recently been made in using transgenic animals as bioreactors to produce large quantity and high quality pharmaceuticals. The overall strategy entails the use of tissue-specific promoters to drive the expression of genes encoding medically important molecules. When those molecules are expressed in the target tissue of

transgenic animals and secreted into body fluids, they can be harvested, purified and used for treating human diseases. The most notable example is the milk-based bioreactor system, taking advantage of mammary gland-specific gene promoters. U.S. Patent No. 5,476,995 was one of the first patents directed to transgenic female sheep as milk-based bioreactors that expressed the transgene in the mammary gland so as to produce the target protein in its milk.

A number of proteins have been produced in milk-based bioreactor systems, such as protein C (U.S. Patent No. 5,589,604), blood coagulation factors (U.S. Patent No. 5,322,775), fibrinogen (U.S. Patent No. 5,639,940), antibodies (U.S. Patent No. 5,625,126) and hemoglobin (U.S. Patent No. 5,602,306), some of which are now being used in clinical trials. However, even in view of its initial success, a milk-based bioreactor system has several limitations. The first relates to its relatively low degree of cost-effectiveness. For instance, the lactation of transgenic livestock does not occur until an average of one and a half years old. Besides, lactation only occurs in female animals and lasts for a limited period of time. Secondly, purification of target proteins from milk often requires the development of complicated purification schemes (Wilkins et al, 1992). Thirdly, leakage of biologically active proteins from the mammary gland into the blood stream commonly occurs with the possibility of leading to pathological conditions in transgenic animals.

Another potential bioreactor system that can circumvent some of the above-mentioned limitations is a urine-based system where urine is an easily collectable fluid from transgenic livestock animals. This bioreactor system has been recently tested by Kerr and colleagues (1998), among whom is one of the present inventors, in transgenic mice using a urothelium-specific promoter (uroplakin II promoter) to drive human growth hormone (hGH) expression and production. They found that hGH could indeed be found in the urine of these transgenic mice at a concentration of 0.1 mg/ml, indicating that the urothelium can serve as an alternate bioreactor. The major advantages of this urine-based system over milk-based systems are the ability to harvest the product soon after birth and throughout the life of the animal irrespective of sex or reproductive status and the ease of product purification from urine. In addition, livestock urine is a proven, currently utilized source of pharmaceuticals; it is estimated that urine is being collected from 75,000 pregnant horses annually as a source of estrogenic compounds for postmenopausal hormone replacement therapy (Williams, 1994).

Despite these major advantages, several technical problems still exist with the above-mentioned urine-based bioreactor system, the most important being the relatively low yield of urinary hGH (0.1 mg/ml) obtained by Kerr et al (1998), as most of the hGH appear to be trapped in the cytoplasm of the superficial urothelial cells. This relatively low yield may be because the urothelium is not known to be a major secretory epithelium and the purification

of a minor protein from urine may require sophisticated purification procedures. In addition, low levels of hGH was found to have leaked into the mouse blood stream, possibly being responsible for the infertility observed in the transgenic female mice.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome the above-mentioned deficiencies in the art by providing a urine-based bioreactor system using a kidney-specific promoter for the expression and production of a recombinant biologically active polypeptide and a targeting system for the apical surface membrane of kidney epithelial cells.

The present invention provides a recombinant DNA molecule containing a kidney-specific promoter operably linked to a heterologous DNA sequence, which kidney-specific promoter is capable of expressing the heterologous biologically active polypeptide, encoded by the heterologous DNA sequence and containing an apical membrane targeting system, in the kidney of a host animal to produce a recombinant biologically active polypeptide in the urine.

As an embodiment of the present invention, the heterologous biologically active polypeptide contains a glycosyl phosphatidylinositol (GPI) signal sequence at its C-terminus to target the heterologous biologically active polypeptide to the apical surface of kidney epithelial cells for secretion into the lumen. In another embodiment, the heterologous biologically active polypeptide can be expressed as a fusion polypeptide between a biologically active polypeptide of interest and uromodulin via a protease-sensitive linker. The C-terminus of this fusion polypeptide is the C-terminus of uromodulin and contains a GPI signal sequence.

A further embodiment of the present invention provides for introducing one or more non-native sites for glycosylation into the heterologous biologically active polypeptide.

Yet another embodiment of the present invention is directed to an operable linkage of the kidney-specific promoter to both the heterologous DNA sequence encoding a heterologous biologically active polypeptide and a DNA sequence encoding phosphatidylinositol-specific phospholipase C (PIPLC), which DNA sequence encoding PIPLC is positioned downstream from the heterologous DNA sequence relative to the kidney-specific promoter.

A further object of the present invention provides a urine-based bioreactor system in which apical surface membrane targeting is enhanced by the inactivation or deletion of

basolateral surface membrane targeting signals in the recombinant biologically active polypeptide.

The present invention also provides for a method for producing a recombinant biologically active polypeptide *in vivo* using a urine-based bioreactor system in transgenic animals. Further provided are transgenic animals, all of whose somatic cells and preferably all of whose germ cells contain a recombinant construct or transgene from which a biologically active polypeptide is produced in recoverable amounts in the urine.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing the apical and basolateral surfaces of kidney epithelial cells in relation to the urine space (lumen) and blood vessels.

Figure 2 is a schematic diagram showing an embodiment of a GPI-containing construct. The construct contains, from 5' to 3', the uromodulin promoter, hGH gene, and an in-frame GPI signal sequence followed by a stop codon and polyadenylation signal.

Figure 3 is an amino acid sequence comparison/alignment of rat (SEQ ID NO:38), mouse (SEQ ID NO:39), human (SEQ ID NO:40), and bovine (SEQ ID NO:41) uromodulin. Boxes represent potential Asn-linked glycosylation sites and underlines represent the GPI attachment site and indicate that the sequence in this GPI attachment site of uromodulin is highly conserved across species.

Figure 4 shows a restriction digestion of five phage clones (lanes 1-5) on agarose gel electrophoresis. M represents lanes of molecular weight markers.

Figure 5 shows a Southern blot corresponding to the agarose gel shown in Fig. 4 hybridized separately with each of the 5'-end, middle region, and 3'-end probes.

Figure 6 shows an agarose gel electrophoresis of PCR reaction products using the sets of primers for the 5'-end, the middle region, and the 3'-end of the uromodulin gene.

Figures 7A and 7B show agarose gel electrophoresis (Fig. 7A) of EcoRI restriction digests of genomic DNA from various animal species and Southern blot hybridization (Fig. 7B) of the restriction digested genomic DNA with the middle region probe.

Figure 8 is a schematic representation of the uromodulin (THP) gene structure in the human, bovine and rat genome. The open boxes represent exons with the exon numbering provided, and the thick bars represent the introns, the lengths of which are variable.

Figure 9 shows Southern blot hybridization of BAC plasmid clone 1 digested with the restriction enzymes, PstI (lane 4), ApaI (lane 6), EcoRI (lane 7), SacI (lane 8), and KpnI (lane 10) and hybridized separately with 5'-end, middle region and 3'-end probes.

Figures 10A-10H show the nucleotide sequence of the mouse uromodulin promoter region (SEQ ID NO:1) which is 9,345 bp upstream of the first mouse uromodulin coding exon.

Figure 11 is a schematic presentation of the mouse uromodulin promoter in which the arrow denotes the transcription initiation site, the letters denote restriction sites (A, ApaI; P, PstI; B, BamHI; H, HindIII; S, SpeI), and the short bar denotes the relative size of the DNA.

Figure 12 shows the partial cDNA sequence of goat uromodulin gene (SEQ ID NO:2). The location of primers AS14, AS15 and AS17 used for isolation of goat uromodulin genomic DNA is shown in underline.

Figure 13A and 13B show the nucleotide sequence of goat uromodulin gene intron 1 (Fig. 13A; SEQ ID NO:3) and exon 3 (Fig. 13B, SEQ ID NO:4). The location of primers AS1, AS2, AS3, AS4 and AS5 used in genomic walking is indicated.

Figures 14A and 14B show the nucleotide sequence of the goat uromodulin promoter region (SEQ ID NO:37). The boxed sequence denotes the TATA box and the arrow denotes the putative transcription initiation start site.

Figures 15A and 15B show a homology comparison of goat and mouse uromodulin promoter regions corresponding to nucleotide positions 1121-1629 in SEQ ID NO:37 and nucleotide positions 6679-7191 in SEQ ID NO:1 (designated in Figs. 15A and 15B as nucleotides 6677-7189), respectively. Gaps are denoted by a period (.) between nucleotides.

Figure 16 is a schematic diagram illustrating the construction of chimeric gene with a mouse uromodulin promoter and the coding sequence of human growth hormone. A 3.0 kb 5'-upstream sequence of the mouse uromodulin gene was cloned upstream of a 2.1 kb human growth hormone coding sequence.

Figure 17 shows a Southern blot analysis of mouse tail DNA of founder mice. Lanes 1 and 5 are non-transgenic control mice showing the endogenous fragment (Endo) of uromodulin coding sequence that hybridized with the uromodulin probe.

Figure 18 shows the results of a radioimmunoassay in the detection of hGH in the urine of transgenic mice.

Figure 19 shows a comparison of the urine and serum concentrations of hGH in transgenic mice.

Figure 20 shows a radioimmunoassay recovery test of hGH in test mice.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the development of a bioreactor system in a transgenic mammal where a recombinant biologically active polypeptide is produced and secreted into the urine by the kidney-specific expression of a heterologous polypeptide, which is encoded by a heterologous DNA sequence, under the direction of a kidney-specific promoter, such as the uromodulin promoter. This urine-based mammalian bioreactor system, according to the present invention, is obtained by producing a transgenic mammal in which an isolated DNA molecule containing a recombinant construct or "transgene" for kidney- specific expression and production of the biologically active protein of interest is stably introduced. An example of a urine-based bioreactor system where the protein of interest is expressed in urothelial cells, rather than kidney cells, but which serves as guidance to development of a urine-

based bioreactor system, is provided by Lin et al (1995) and Kerr et al (1998). The present invention advantageously combines kidney-specific expression with apical surface membrane targeting to overcome the problems associated with leakage of an expressed heterologous biologically active polypeptide into the bloodstream.

To produce transgenic animals, any method known in the art for introducing a recombinant construct or transgene into an embryo, such as microinjection, cell gun, transfection, liposome fusion, electroporation, and the like, may be used. However, the most widely used method for producing transgenic animals, and the method most preferred according to the present invention, is microinjection, which involves injecting a DNA molecule into the male pronucleus of fertilized eggs (Brinster et al, 1981; Costantini et al, 1981; Harbers et al, 1981; Wagner et al, 1981; Gordon et al, 1976; Stewart et al, 1982; Palmiter et al, 1983; Hogan et al, 1986; U.S. Patent No. 4,870,009; U.S. Patent No. 5,550,316; U.S. Patent No. 4,736,866; U.S. Patent No. 4,873,191). While the above methods for introducing a recombinant construct/transgene into mammals and their germ cells were originally developed in the mouse, they were subsequently adopted for use with larger animals, including livestock species (WO 88/00239, WO 90/05188, WO 92/11757; and Simon et al, 1988). Microinjection of DNA into the cytoplasm of a zygote can also be used to produce transgenic animals.

Alternatively, a recombinant construct or transgene can be introduced into embryonic stem cells (ES cells) by any

method known in the art, such as those identified above as non-limiting examples. The ES cells transformed with the transgene are combined with blastocyst of the same animal species to colonize the embryo (Jaenisch, 1988). In some embryos, these transformed ES cells form the germline of the transgenic animal generated by this procedure. Transformed ES cells can also be used as a source of nuclei for transplantation into an enucleated fertilized oocyte to produce a transgenic animal.

The present invention for producing a biologically active polypeptide in a urine-based mammalian bioreactor system is not limited to any one species of animal, but provides for any appropriate non-human mammal species. For example, while mouse is a mammal species that is routinely used for producing transgenic animals and, thus, serves as a model system to test the transgene, other non-limiting but preferred examples include farm animals, such as pigs, sheep, goats, horses and cattle, which generate large quantities of urine, may be suitably used. A most preferred animal for use as a urinary bioreactor is a goat.

The success rate for producing transgenic animals by microinjection is highest in mice, where approximately 25% of fertilized mouse eggs into which the DNA has been injected, and which have been implanted in a female, will develop into transgenic mice. Although a lower success rate has been achieved with rabbits, pigs, sheep and cattle (Jaenisch, 1988; Hammer et al, 1985 and 1986; Wagner et al, 1984), the production of transgenic livestock is considered by those in

the art to be routine and without undue experimentation. Wall et al (1997a), Velander et al (1997), Drohan (1997), Hyttinen et al (1994), Morcol et al (1994), Lubon et al (1997), Houdebine (1997), Wall et al (1997b), Van Cott et al (1997), Cameron (1997), Cameron et al (1994), Niemann (1998) and Hennighausen (1992), among others, have reported and discussed the use of livestock as bioreactors or factories for the production of biologically active proteins.

The introduction of a DNA containing a transgene sequence at the fertilized oocyte stage ensures that the introduced transgene will be present in all of the germ cells and somatic cells of the transgenic animal. The presence of the introduced transgene in the germ cells of the transgenic "founder" animal, in turn, means that all of the founder animal's offspring will carry the introduced transgene in all of their germ cells and somatic cells.

There is no need for incorporating any plasmid or viral sequences with the gene being introduced, (Jaenisch, 1988), although the vector sequence may be useful in some instances. In many cases however, the presence of vector DNA has been found to be undesirable (Hammer et al, 1987; Chaka et al, 1985 and 1986; Kollias et al, 1986; Shani 1986; Townes et al, 1985). For instance, the transgene construct can be excised from the vector used to amplify the transgene in a microbial host by digestion with appropriate restriction enzymes. The transgene is then recovered by conventional methods, such as electroelution followed by phenol extraction and ethanol precipitation, sucrose density gradient

centrifugation, chromatography, HPLC, or combinations thereof. It has been reported in U.S. Patent No. 5,589,604 that high transformation frequencies, on the order of 20% or more, in both mice and pigs were obtained by microinjection with HPLC-purified DNA.

In order for the introduced gene sequence to be capable of being specifically expressed in the kidney of the transgenic animal, the gene sequence must be operably linked to a kidney-specific promoter. A DNA molecule is said to be "capable of expressing" or "capable of directing the expression of" a polypeptide if it contains nucleotide sequences which contain cis-acting transcriptional regulatory information, and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression. The cis-acting regulatory regions needed for gene expression in general include a promoter region, and such regions will normally include those 5'-non-coding sequences involved with initiation of transcription. A promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. Thus, the DNA sequence encoding a polypeptide of interest is operably linked to a kidney-specific promoter to generate a recombinant construct or "transgene" that is then introduced into the fertilized embryo or ES cells.

Also included in the transgene are nucleotide sequences that encode the signal sequences that direct secretion of the expressed biologically active polypeptide of interest into the urine of the transgenic animal. Both endogenous and heterologous signal sequences (either for the host or for the biologically active protein of interest) can be used, although the endogenous signal sequence of the heterologous protein of interest is preferred. Furthermore, other regulatory sequences in addition to the promoter, such as enhancers, splice signals, ribosome binding sites and polyadenylation sites, etc., may be useful in the transgene construct as would be well-recognized by those of skill in the art.

The preferred promoter in the recombinant construct/ transgene for the kidney-specific expression of a heterologous biologically active polypeptide of interest is the promoter for uromodulin. Uromodulin, also named Tamm-Horsfall protein (THP), is by far the most abundant urinary protein of human and other higher mammals, with an excretion rate of up to 200 mg per day (Hunt et al, 1985; Reinhart et al, 1989). This ~90 kDa glycoprotein has several important features that are relevant to its use in a kidney-expressed urine-based bioreactor system. The protein is synthesized by the epithelial cells of the ascending limb of Henle's loop and the beginning portion of the distal convoluted tubule, delivered exclusively to apical membrane and secreted into the urine (Sikri et al, 1981; Bachmann et al, 1990). Rindler et al (1990) established that uromodulin is a cell surface protein

anchored onto the apical plasma membrane via a glycosylphosphatidyl inositol (GPI) tail, where phosphatidylimositol-specific phospholipase C (PIPLC) cleavage *in vitro* of the GPI linkage completely releases the molecule into the culture medium.

Uromodulin is highly tissue-specific, being expressed only in the kidneys and not in any other epithelial and mesenchymal tissue. Moreover, uromodulin is evolutionarily conserved throughout placental animals. The cDNA sequences reported for rat uromodulin (Fukuoka et al, 1992) and human uromodulin (Hession et al, 1987; Pennica et al, 1987) were found to be 91% and 77% identical with the mouse uromodulin cDNA sequence, respectively (Prasadan et al, 1995). Prasadan and colleagues (1995) also reported that an alignment of uromodulin amino acid sequences from mouse, rat and human showed 91% similarity and 86% identity between mouse and rat, and 79% similarity and 70% identity between mouse and man.

As discussed in the Example 1 presented herein, the laboratory of the present inventors has isolated and sequenced a 9,345 base pair region including about 7 Kb upstream of the coding region of the mouse uromodulin gene, which region contains the mouse uromodulin promoter. This DNA promoter region, or a fragment thereof which retains the tissue specific promoter activity thereof, is used for construction of a transgene with a biologically active polypeptide of interest, i.e., human growth hormone (hGF). While knowledge of the nucleotide sequence of the mouse uromodulin promoter

would facilitate the construction of a transgene which is capable of kidney-specific expression of a biologically active polypeptide of interest, such sequence information is not necessary because it is well within the skill of the art to isolate a functional promoter sequence given a uromodulin genomic clone with the upstream promoter region. There is a wealth of scientific literature directed to the isolation and identification of a promoter for a given gene, with the Kahari et al (1990) article on the delineation of functional promoter and regulatory cis-elements being just one representative citation. Clones containing the goat uromodulin gene promoter have also been obtained as disclosed herein in Example 2 with the sequence of the goat uromodulin promoter being presented in Figs. 11A and 11B. Other uromodulin gene promoters can be further isolated using the genomic walking procedure described for the isolation of the mouse and goat uromodulin gene promoters in the Examples herein.

As a preferred embodiment of the present invention, a uromodulin-based urine bioreactor system has the following advantageous features:

(1) Uromodulin is a kidney-specific and abundantly expressed gene and its synthesis is confined to the thick-ascending limb of Henle's loop and early distal tubules of the kidneys. Biologically important genes under the control of uromodulin promoter are likely to be expressed in the same location and secreted into the urine, where the expressed gene products can be readily purified.

(2) Year-round production, which is independent of age and sex as compared to mammary-based bioreactor.

Uromodulin has already been reported to be evolutionarily conserved, being detectable immunologically in all placental mammals (Kumar et al, 1990). The laboratory of the present inventors has shown by Southern blot hybridization that the uromodulin gene is present as a single copy in many mammals, including all important livestock, such as cattle, sheep, goat, horse and pig. Not only do the uromodulin cDNAs from human, mouse and rat share a high level of identity (on the order of 80% or more), but even the high mannose glycosylation of uromodulin is highly conserved among different species of mammals. This strongly suggests that the promoter sequences of uromodulin are also likely to be conserved among mammals.

Moreover, as evidenced by the numerous examples in the scientific literature of promoters that are interchangeable among species, the uromodulin promoter from one mammal species is believed to be functional in another species. Accordingly, the mouse uromodulin promoter identified herein may be able to be used directly in transgenic livestock to drive kidney-specific expression of the biologically active polypeptide of interest in a urine-based bioreactor system. Alternatively, the uromodulin promoter used in the transgenic livestock to drive kidney-specific expression of the biologically active polypeptide can be its own endogenous uromodulin promoter, such as using the goat uromodulin to drive kidney-specific expression in

transgenic goats, or an interchangeable uromodulin promoter from another species of livestock. A computer comparison of the nucleotide sequences of the goat and mouse uromodulin promoter regions determined by the laboratory of the present inventors only found homology (approximately 74%) over a short stretch of about 500 bp that includes the first exon of uromodulin (Fig. 12A). No other significant homology was found within about 1,100 bp of the promoter region 5'-upstream of this short stretch of homology. If it is later determined that the mouse promoter or any other non-native uromodulin promoter does not provide sufficiently kidney-specific expression in the transgenic animal, then the native uromodulin promoter would be used instead in the transgene construct.

The bovine and rat uromodulin promoter regions have already been identified in Yu et al (1994), the entire contents of which are hereby incorporated herein by reference. Specifically, Fig. 5 of Yu et al (1994) shows the nucleotide sequence of the bovine and rat uromodulin promoter regions. These promoter regions, or a fragment thereof with kidney-specific promoting activity, can be used to drive the kidney-specific expression of a heterologous gene in those respective species. If it is determined that the regions of the approximately 600 base pairs upstream of the transcription start site in the bovine and rat sequences of Fig. 5 of Yu et al (1994) do not contain the complete kidney-specific uromodulin promoter sequence for these species, additional nucleotides upstream of the disclosed sequences can readily be

obtained and sequenced using the specific sequences as a probe of bovine and rat genomic libraries, or using the technique of genomic walking as described in the examples herein, without the use of undue experimentation.

Uromodulin promoters from other mammalian species can be isolated using the same approaches outlined in the examples provided herein, or using the same approach used in Yu et al (1994), or by hybridization or PCR amplification of genomic libraries or genomic DNAs using probes or primers from the genomic clones of the mouse, goat, rat or cow uromodulin gene. If the need to use a uromodulin promoter from another livestock animal species arises, then information generated from the mouse and goat uromodulin promoters or from the bovine and rat uromodulin promoter region of Yu et al (1994) can be used to facilitate this process. For instance, as the sequence of the mouse and goat uromodulin promoters have now been determined and are reported herein, and the bovine, rat and human promoter regions have been previously reported, oligonucleotide primers based on these sequences can be designed for PCR reactions. Long-range PCR can be performed to directly isolate uromodulin promoters from a pool of genomic DNAs extracted from various livestock animal species. DNA fragments containing the uromodulin promoter from livestock animal species can also be identified by hybridization of genomic libraries of corresponding species with mouse, goat, bovine, rat or human uromodulin promoter probes under hybridization conditions similar to or the same

as that used for the Southern blots (Zoo-blots of genomic DNA from various species) disclosed in Example 1 provided herein.

As will be appreciated by those in the art, the uromodulin promoter or any other kidney-specific promoter used in the transgene for directing kidney-specific expression of the biologically active polypeptide of interest can include relatively minor modifications, such as point mutations, small deletions or chemical modifications that do not substantially lower the strength of the promoter or its tissue-specificity.

In addition, the identification of additional promoters active in directing gene expression in the kidney can be routinely performed using the suppression subtraction hybridization library technique. Using this technique, which eliminates the cDNAs that are shared by multiple tissues (Diatchenko et al, 1996), a library highly enriched in kidney-specific cDNAs can be generated. Total RNAs are isolated from stomach, intestine, colon, liver and brain, and Northern blot analysis of these mRNAs using an actin cDNA as a probe is used to demonstrate the intactness of the actin mRNA in all of these preparations. Kidney cDNAs are then used as the "tester", and the cDNAs of all the other non-kidney tissues, referred to as the "drivers", are subtracted from the kidney cDNAs. Using the subtraction library technique, the laboratory of the present inventors had earlier probed the cDNAs of the non-subtracted and the subtracted libraries with actin cDNA or uroplakin Ib cDNA, and the results indicated that the original (non-subtracted) bovine bladder cDNA

preparation contained abundant actin mRNA and relatively little uroplakin Ib mRNA. In contrast, the subtracted library contained almost no detectable actin mRNA (at least 50 fold reduction) but greatly increased uroplakin Ib mRNA (>10 to 15 fold enrichment). Multiple cDNA clones have been isolated from the subtraction library and used to probe the mRNAs of various bovine tissues. For example, a uroplakin Ib probe confirmed its bladder specificity.

The laboratory of the present inventors have already been successful in obtaining three unidentified cDNAs in which the tissue distribution pattern showed bladder specificity. Sequencing data indicate that these three bladder-specific clones are novel genes not described previously. In the same manner, kidney-specific genes can be isolated, and any gene that is involved in the structure and function of the excretory tract of the kidney, including proximal, distal tubules, Henle's loop, collecting duct system can be applied in this system to isolate its promoter for use in expressing and producing a biologically active protein in a urine-based kidney bioreactor. Although the suppression subtraction hybridization library technique is the preferred procedure for obtaining tissue-specific genes, kidney-specific genes can also be identified through other well-known methods, including biochemical methods, protein chemistry, monoclonal antibody production, two-dimensional gel electrophoresis, cDNA library screening, expression library screening, differential display, phage display, etc.

Although there is an abundance of evidence suggesting that many important regulatory elements are located 5' to the mRNA cap site (McKnight et al., 1982; Payvar et al., 1983; Renkowitz et al., 1984; Karin et al., 1984) and in a great majority of cases the 5'-flanking region is sufficient to convey the tissue-specificity and high-level expression of a tissue-specific gene, it has been reported that in some instances important regulatory elements, particularly those mediating tissue-specific expression, may reside within the structural gene, i.e., introns, or even the 3'- to it in the untranslated sequences, and contribute to promoter activity (Charnay et al., 1984; Gillies et al., 1983; Sternberg et al., 1988). For example, intron I sequences were found to be important for high-level and tissue-specific expression of an α-skeletal actin gene, a β-globin gene and a peripherin gene (Reecy et al, 1998; James-Pederson et al, 1995; Belecky-Adams et al, 1993). In view of these examples of introns or 3'-untranslated sequences contributing to promoter activity, the constructs to be made may include intron I sequences of a kidney-specific gene and, when necessary, 3'-untranslated sequences placed downstream of the DNA sequence encoding the heterologous polypeptide of interest according to the present invention. In the former case, a fragment will be isolated that spans the 5'-flanking region, the first exon and the first intron, followed by the DNA sequence encoding the biologically active polypeptide of interest. The translation initiation codon of the kidney-specific gene could also be mutated to avoid translation of a truncated protein, and other

regions of the kidney-specific gene could also be used to ensure the tissue-specific and high-level expression of the transgene.

As used herein, "biologically active polypeptide" refers to a polypeptide/protein capable of causing some effect within an animal and preferably not within the animal having the transgene. Examples of such polypeptides/proteins include, but are not limited to, adipokinin, adrenocorticotropin, blood clotting factors, chorionic gonadotropin, corticoliberin, corticotropin, cystic fibrosis transmembrane conductance regulators, erythropoietin, folliberin, follitropin, glucagon gonadoliberin, gonadotropin, human growth hormone, hypophysiotropic hormone, insulin, lipotropin, luteinizing hormone-releasing hormone, luteotropin, melanotropin, parathormone, parotin, prolactin, prolactoliberin, prolactostatin, somatoliberin, somatotropin, thyrotropin, tissue-type plasminogen activator, vasopressin, antibodies, peptides, and antigens (for use in vaccines). It will be appreciated by those of skill in the art that the above list is not exhaustive. In addition, new genes for biologically active proteins that will function in the context of the present invention are continually being identified.

Proteins which degrade or detoxify organic material may also be produced by means of the present invention. Such proteins may be those discussed in WO 99/28463, the entire contents of which is hereby incorporated by reference.

The biologically active polypeptide produced in the urine-based bioreactor system according to the present

invention can be isolated from the urine of these transgenic animals. Accordingly, the present invention provides a means for isolating large amounts of biologically active polypeptides from the urine of transgenic animals which can be used for a variety of different purposes. Furthermore, the biologically active polypeptide can be readily recovered and purified from the urine as would be well within the skill of those in the art.

Because the uromodulin promoter is a preferred promoter for the kidney-based urinary bioreactor system according to the present invention, a transgenic mouse model, in which a mouse uromodulin promoter is operably linked to a DNA sequence encoding human growth hormone, was generated. As described in Example 3, a transgene containing a 3.0 kb mouse uromodulin promoter and 2.1 kb human growth hormone gene was constructed and injected into the fertilized eggs of FVB/N inbred mice. Out of the 42 live-born animals, three animals carried the transgene as evidenced by the appearance of a 5.1 kb transgene fragment in Southern blot hybridization of tail DNA. Upon radioimmunoassay, two of these founder mice were found to secret human growth hormone into the urine. Unexpectedly however, one of the two positive mice that secreted the human growth hormone died at 4 months of age. The remaining positive mouse showed, in addition to urinary hGH, a high concentration of hGH in the serum. These observations, together with the result that the remaining positive male mouse failed to impregnate two batches of female mates strongly indicate that the leakage of hGH into the serum

inadvertently affected the physiology and reproductive ability of the founder animals.

Although the adverse effects of leakage of biologically active molecules into the bloodstream have been well documented in the transgenic bioreactor field, definite solutions are scarce, if not nonexistent. The leakage into the bloodstream in transgenic animals can result in severe consequence including the loss of capacity of the bioreactor, rendering it inefficient or inoperable. In the case of urine-based bioreactor, the yield of hGH is compromised; the leakage of hGH into the bloodstream leads to premature death and infertility of the animals. The success of this bioreactor system therefore largely depends upon whether the leakage problem can be solved.

Cell membranes in polarized epithelial cells are functionally divided into apical and basolateral membranes (FIG.1). The problem of leakage of hGH into the bloodstream is due to the non-directed secretion of hGH into both the apical surface and the basolateral sides of the membrane which are in close vicinity to blood vessels underlying the epithelial cell layer. A unique aspect of the present invention is directed to apical membrane targeting and urinary secretion of the recombinant proteins, which apical targeting minimizes basolateral leakage of the biologically active polypeptide of interest into the bloodstream and thereby also increasing the amount of hGH being secreted into the urine. When a recombinant polypeptide is targeted to the basolateral surface or lacks an apical targeting signal, this protein can be

easily leaked into the blood, potentially causing pathological conditions in transgenic animals. Enhanced apical targeting in uromodulin-synthesizing cells will overcome this problem because the recombinant polypeptide will be directly released into the urinary space.

While a great majority of cellular proteins are either secreted or permanently anchored onto the cell membrane, a small group of proteins are temporarily anchored onto the external surface of the plasma membrane via glycolipids. These anchors are termed glycosyl phosphatidylinositols (GPIs) and cleavage of the GPI by phospholipases can release the protein from the membrane. Although the exact function of the GPI linkage is unclear, one of the proposed functions for a GPI sequence is the possibility that GPI serves as an apical targeting signal. A GPI signal sequence usually contains two parts: a stretch of 17-30 hydrophobic amino acids at the very end of the C-terminus of a protein, which will be cleaved and thus be absent in mature proteins, and a shorter stretch (about 8-14 amino acids) containing small amino acids and serving as the GPI anchorage site. GPI structure and the biosynthesis of GPI anchored membrane proteins are reviewed in Englund (1993) and Udenfriend et al. (1995).

According to a preferred embodiment of the present invention, apical surface membrane targeting is provided by a GPI signal sequence. Therefore, in the present invention, a kidney-specific promoter, preferably the uromodulin promoter, drives the expression of a gene or cDNA encoding a recombinant

polypeptide with a GPI signal sequence placed at its C-terminus. This transgene construct will be achieved by constructing from 5'-end to 3'-end, a uromodulin promoter, a DNA sequence encoding a recombinant polypeptide, and a DNA sequence encoding a GPI signal (Fig. 2). This will allow the production of a recombinant polypeptide whose C-terminus is modified with a GPI signal sequence which will be linked with GPI. The GPI sequence can also be located at the N-terminus of the recombinant polypeptide or in the middle of a protein or a fusion protein. With the GPI as an apical targeting signal, the heterologous polypeptide is directed exclusively to the apical surface, instead of to both the apical and basolateral surfaces, where the heterologous polypeptide anchored to the apical membrane will be released into the urine by the action of PIPLC enzyme.

Figure 3 shows an amino acid alignment/comparison of rat, mouse, human, and bovine uromodulin. At the C-terminus, GPI signal sequence of rat (SEQ ID NO:42), mouse (SEQ ID NO:43), human (SEQ ID NO:44) and bovine (SEQ ID NO:45) are aligned and compared. The underlined sequences denote the GPI attachment site with the GPI addition site most likely being serine. It is clear that there is cross-species conservation of the GPI signal sequences between rat, mouse, human and bovine uromodulin.

Although the GPI signal sequence of uromodulin (THP) is preferred in the transgene construct according to the present invention because uromodulin is naturally targeted to the apical surface and because the uromodulin GPI signal

sequence is known to be efficiently cleaved *in vivo*, GPI signal sequences of other proteins, such as *Torpedo* acetylcholinesterase (Sikorav et al., 1988; SEQ ID NO:46), placenta alkaline phosphatase (Micanovic et al., 1988; SEQ ID NO:48), *T. brucei* PARP (Clayton et al., 1989; SEQ ID NO:49), hamster prion protein (Stahl et al., 1990; SEQ ID NO:50), rat Thyl (Seki et al., 1985; SEQ ID NO:51), *T. brucei* VSG (Boothroyd et al., 1980; SEQ ID NO:52), etc., can be suitably used. In the above-mentioned GPI signal sequences, GPI anchor addition involves the removal of residues C-terminal to residue 13 of SEQ ID Nos: 46-52. It should be noted that even though the GPI signal sequences of these other GPI anchored proteins are not highly sequence conserved, they have structural features that suffice for attachment of the GPI anchor.

An outline of a method for constructing a chimeric polypeptide containing a heterologous polypeptide of interest and GPI signal sequence at its C-terminus is as follows:

1) Creation of a restriction cloning site before the stop codon of hGH by site-directed mutagenesis using the vector containing the uromodulin-hGH construct described in Example 3.

2) Generation of cDNA fragment encoding a GPI-consensus sequence, preferably using the GPI signal sequence of uromodulin. PCR will be performed to amplify a DNA fragment encoding a GPI signal sequence. A restriction cloning site that is identical to the site before the stop codon within hGH will be incorporated into the PCR primers to facilitate

cloning, with caution being exercised to ensure that the GPI signal sequence is in the correct translational reading frame with the hGH sequence.

3) Cloning of the DNA fragment encoding the GPI signal sequence into the hGH-encoding DNA sequence.

4) Generation of transgenic mice producing hGH in the urine.

Based on a suitable GPI signal sequence, a universal GPI cassette that is applicable for cloning of a GPI signal sequence at the C-terminus of most, if not all, biologically active polypeptides can be constructed.

An alternative strategy for enhancing the apical secretion of recombinant polypeptides in urine-based kidney bioreactor is to produce a fusion protein between a desired polypeptide and uromodulin. This can be accomplished by constructing a DNA sequence containing the cDNA or gene encoding the desired polypeptide followed by a chemically or enzymatically cleavable linker sequence such as a protease-sensitive linker sequence (e.g., thrombin-sensitive sequence) and by a uromodulin cDNA sequence. This approach has several major advantages. First, since the endogenous uromodulin is predominantly targeted to the apical surface membrane, uromodulin can serve as a carrier for bringing the recombinant heterologous polypeptide to the apical surface. Second, since uromodulin has a tendency to form large, stable aggregates in the urine, the fused polypeptide will likely be more stable in aggregates than as a soluble polypeptide. Third, the aggregated fused polypeptide can be readily purified by first

centrifuging the urine to obtain the aggregates, and then cleaving the away the uromodulin portion by using a protease such as thrombin.

The release of uromodulin from the apical membrane into the urine requires the action of the PIPLC enzyme which specifically cleaves the GPI linkage. Likewise, the release of the GPI-linked recombinant polypeptide or recombinant polypeptide-uromodulin fusion protein in uromodulin-synthesizing cells would require a similar mechanism. Although, at the luminal surface of uromodulin-synthesizing cells, there naturally exists functional PIPLC, the amount of the enzyme may not be sufficient to handle large amounts of recombinant polypeptides with a GPI signal sequence. In this respect, overexpression of PIPLC under the direction of a kidney-specific promoter, preferably a uromodulin promoter, will ensure a sufficient amount of PIPLC to efficiently release GPI-anchored recombinant polypeptides from the apical surface. To do this, two constructs, one encoding the recombinant heterologous polypeptide and the other encoding PIPLC, could be co-injected into fertilized eggs to produce an animal bi-transgenic for the recombinant heterologous polypeptide and PIPLC. More likely however, two separate types of transgenic animals instead of a bi-transgenic animal are generated, one of which expresses the recombinant heterologous polypeptide of interest and the other expresses PIPLC. Bi-transgenic animals can then be readily produced by cross-breeding the two separate types of transgenic animals.

Another embodiment of apical surface membrane targeting according to the present invention is to make use of glycosylation of polypeptides as an apical targeting signal. Asn-linked glycosylation has been thought to be a facilitator of apical targeting signal for soluble and membrane proteins in epithelial cells. Although the mechanism is unclear, it has been hypothesized that the glycosylation may serve to interact with lectin-like molecules that are strategically located along the pathway toward the apical surface membrane. By adding one or more non-native glycosylation consensus sequences to a polypeptide which otherwise does not contain a glycosylation site (such as human growth hormone), one could achieve glycosylation, and thereby enhance apical targeting of the polypeptide. The glycosylation consensus sequence is the three amino acid sequence, Asn-Xaa-Ser/Thr, where Xaa can be any amino acid with the exception of proline and aspartic acid. To minimize the number of the amino acid substitutions in a given sequence, a strategy can be employed to introduce a non-native glycosylation site at a sequence containing Asn-Xaa-Xaa (the second Xaa being any amino acid other than Ser/Thr) to Asn-Xaa-Ser/Thr. Alternatively, an original sequence containing Xaa-Xaa-Ser/Thr can be changed to Asn-Xaa-Ser/Thr. To maximize the likelihood of the site being glycosylated, the sites will be designed at β-turns in the structure of the polypeptide, where such non-native sites will have a greater chance of being glycosylated. Globally, the glycosylation consensus sequence can be located at the N- or C-terminus or in the middle of the polypeptide, provided that

the mutation of a single amino acid does not impair the original biological function of the polypeptide. For any of the above-mentioned strategies for introducing a glycosylation consensus sequence, any method of site-directed mutagenesis can be performed on cDNA or gene encoding the polypeptide. In order to change a codon encoding any amino acid to Asn (AAU/C), a maximum of 3 point mutations, which can be easily accomplished by routine site-directed mutagenesis, would be required.

In addition to Asn-linked glycosylation, O-glycosylation has been shown to enhance the apical targeting of some epithelial membrane proteins. In general, the sites for O-glycosylation are clusters of serines and threonines (Sadeghi et al., 1999). Proline residues adjacent to serine and threonine residues enhances O-glycosylation (Yoshida et al; 1997). For example, the apical targeting of sucrase isomaltase, an intestinal brush border protein, requires the O-glycosylation of a stretch of 12 amino acids (Ala(37)-Pro (48)) juxtaposed to the membrane anchor. Yoshida et al. (1997) also reported that a sequence stretch containing Xaa-Thr-Pro-Xaa-Pro appears to be a good substrate for O-glycosylation. Accordingly, the Xaa-Thr-Pro-Xaa-Pro sequence stretch can also be introduced into the heterologous polypeptide of interest by site-directed mutagenesis.

An alternative strategy to produce a higher level of PIPLC than is normally produced in uromodulin-synthesizing kidney epithelial cells is to construct a DNA molecule in which a DNA sequence encoding PIPLC is placed 3'(downstream

from) of a construct where a kidney-specific promoter is operably linked to a DNA sequence encoding a heterologous polypeptide. The placement of the DNA sequence encoding PIPLC allows the kidney-specific promoter to be operably linked to both the DNA sequence encoding the heterologous protein and the DNA sequence encoding PIPLC. Thus, "bi-cistronic" mRNA can be transcribed from this particular type of construct.

An alternative to apical targeting by the addition of GPI or by glycosylation is the inactivation of potential basolateral targeting signals that are present in the heterologous polypeptide of interest. It has been reported that, in some instances, basolateral targeting depends on a distinctive cytoplasmic targeting signal, for example a tyrosine motif or a di-leucine motif.

The so-called tyrosine motif for basolateral targeting contains a consensus sequence YXXO where the first residue (Y) is tyrosine, the last amino acid (denoted by O) is a bulky hydrophobic amino acid residue (most commonly Leu), and the middle two residues can be any amino acid residue (Deschanbeault et al., 12991; Stephens et al., 1998). A double or di-leucine motif is also important for basolateral targeting. This motif is basically two (double) leucine residues (di-leucine; Hunziker et al., 1994). The tyrosine and di-leucine motifs are found frequently at the C-terminus of the protein or in the cytoplasmic domain of a membrane protein. Deletion or modification of these motifs will likely lead to the blockage of basolateral targeting. Experimental strategies to be employed in this alternative to apical

targeting include the removal of a segment at the C-terminus of a heterologous polypeptide that contains a basolateral targeting signal sequence, or the mutation of tyrosine and di-leucine motifs contained in basolateral targeting signal sequences on the heterologous polypeptide, such as by site-directed mutagenesis of the encoding DNA sequence.

As will be appreciated by those in the art, any combination of the aforementioned targeting approaches can be used. For example, the GPI and glycosylation approaches can be employed simultaneously, or the addition of GPI and/or glycosylation can be combined with the deletion/inactivation of basolateral targeting signal(s). Furthermore, these targeting approaches are not limited to targeting the apical surface membranes of kidney epithelial cells and are believed to also be applicable to other bioreacteor systems such as the mammary gland, urothelial, and seminal bioreactor systems.

While the production of transgenic animals by the introduction of the transgene into germ line cells is most preferred, it is also contemplated that the transgenic animals, which serve as a urinary bioreactor system, can be generated with vectors that are useful for transforming the kidney into a bioreactor capable of producing a biologically active protein in the urine for isolation. The transformed cells may be germ line or somatic cells.

In an alternative embodiment to introduction into germ line cells, the vector according to the present invention includes a system which is well received by the cells lining the excretory tract of the kidney, including proximal, distal

tubules, Henle's loop and collecting duct system. An example of a useful vector system is the Myogenic Vector System (Vector Therapeutics Inc., Houston, TX). In this embodiment, the heterologous DNA sequence encoding the biologically active polypeptide, linked to a viral promoter construct capable of directing kidney-specific expression and carried in the vector, is introduced into the kidney of an animal *in vivo*. Introduction of the vector can be carried out by a number of different methods routine to those of skill in the art. Vectors of the present invention can also be incorporated into liposomes and introduced into the animal in that form. The transgene is absorbed into one or more epithelial cells capable of expressing and secreting the biologically active protein into the urine collecting in the bladder.

Another alternative embodiment for generating a transgenic animal as a kidney-based bioreactor is through the use of targeted homologous recombination, where one copy of the endogenous uromodulin gene is disrupted by insertion of a heterologous gene encoding a biologically active molecule of interest, which heterologous gene is flanked by sequences complementary to the endogenous uromodulin gene. These flanking complementary sequences which direct homologous recombination to an endogenous uromodulin gene are at least 25 base pairs in length, preferably at least 150 base pairs. This technique for generating transgenic animals and cells by homologous recombination is disclosed in WO 90/11354 and U.S. Patent 5,272,071, the entire contents of which are hereby incorporated by reference. Accordingly, if it is desired for

the kidney to express and secrete a selected biologically active polypeptide into the urine, then a short sequence on either side of the start codon of the uromodulin coding sequence in a given species can be used as flanking sequences to create a construct that can be inserted at the specific location in the genome of the host animal species which is between the endogenous uromodulin gene promoter and the endogenous uromodulin gene coding sequence. In this way, the expression of the biologically active polypeptide of interest will be driven by the endogenous uromodulin promoter in the transgenic animal. The bovine genomic uromodulin sequence has already been reported (Yu et al., 1994), and the mouse genomic uromodulin sequence as well as the clone containing the goat genomic uromodulin gene sequence surrounding the start codon are disclosed herein.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLE 1: ISOLATION OF MOUSE UROMODULIN GENE PROMOTER

Generation of Uromodulin cDNA Probes

Three probes corresponding to the 5'-end, the middle region and the 3'-end of the full-length uromodulin cDNA (Prasadan et al, 1995) were generated using the reverse transcription-polymerase chain reaction (RT-PCR) method, with three pairs of oligonucleotide primers chemically synthesized based on the published uromodulin cDNA sequence. The set of

primers for the 5'-end are 5'-TGGACCAGTCCTGTCCTGGTTCAG-3' (SEQ ID NO:5; sense), and 5'-GGGTGTTCACACAGCTGCTGTTGG-3' (SEQ ID NO:6; antisense). The set of primers for the middle region are 5'-AGGGCTTTACAGGGGATGGTTG-3' (SEQ ID NO:7) and 5'-GATTGCACTCAGGGGGCTCTGT-3' (SEQ ID NO:8) The set of primers for the 3'-end are 5'-GGAACTTCATAGATCAGACCCGTG-3' (SEQ ID NO:9) and 5'-TGCCACATTCCTTCAGGAGACAGG-3' (SEQ ID NO:10). These three pairs of oligonucleotide primers were used to amplify uromodulin cDNA fragments using, as a template, a pool of cDNAs reversed transcribed from mouse kidney RNAs. PCR conditions included the first cycle of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; 35 cycles of 95°C for 2 min, 55°C for 1 min, and 72°C for 2 min; and the last cycle of 94°C for 2 min, 55°C for 1 min, and 72°C for 8 min. Agarose gel electrophoresis revealed a 400 bp, a 440 bp and a second 400 bp PCR product for the three sets of primer amplifications, 5'-end, middle region, and 3'-end, respectively. These PCR products were purified by extraction and chromatography using a QIAEX II method (QIAGEN, Valencia, CA).

Screening of Mouse Kidney cDNA Library

A mixture of the above three uromodulin cDNA probes were ^{32}P-labeled and used to screen a BALB/c mouse kidney cDNA library (Clontech, Palo Alto, CA). A total of 2 x 10^5 phage clones from the cDNA library were plated, lifted onto nylon membrane and hybridized with the mixture of probes at 42°C for 16 hours in a solution containing 50% Formamide, 5X SSPE, 5X Denhardt's solution, 0.1% SDS and 100 mg/ml denatured salmon

sperm DNA. After hybridization, the nylon filters were washed at 65°C for 1 hour in 1X SSC and 0.1% SDS, and autoradiographed. Five phage clones were identified from the primary screening, and they were plaque-purified and subjected to the secondary screening using the same conditions as the primary screening. Purified phage clones were amplified by plate lysate and analyzed by EcoRI restriction digestion and agarose gel electrophoresis. On agarose gel, the five clones are of different sizes, ranging from 0.2 kb to 2.7 kb (Fig. 4). A 2.7 kb clone hybridized with all three probes indicating that this band likely represented the full-length mouse uromodulin cDNA clone (Fig. 5). This 2.7 kb band was excised from the bacteriophage with EcoRI restriction enzyme, gel-purified, subcloned into the same site of pBluescript KS$^+$ (Stratagene, LaJolla, CA), and sequenced. The sequence matched precisely with the published mouse (uromodulin cDNA sequence of Prasadan et al, 1995), further establishing the authenticity of this as mouse uromodulin.

Isolation of Mouse Uromodulin Gene

For the isolation of the mouse uromodulin gene, a commercial genomic screening service (Genomic System, St. Louis, MO) was used. Briefly, two pairs of PCR primers located in exon 3 (exon information derived from human uromodulin gene, Pennica et al, 1987) were designed and pre-tested by the present inventors. These primers were then used by Genomic System to mass-screen by PCR pooled genomic (BAC) plasmid clones of the MAC ES Mouse II library which harbors

129/SVJ mouse genomic DNAs. The first pair of primers, sense 5'- AGGGCTTTACAGGGGATGGTTG-3' (SEQ ID NO:11), and antisense 5'- GATTGCACTCAGGGGGCTCTGT-3' (SEQ ID NO:12), was used for the initial screen which yielded two uromodulin clones, each about 60-70 kb in length. These clones were confirmed independently by using a second set of nested primers, sense 5'-GCCTCAGGGCCCGGATGGAAAG-3' (SEQ ID NO:13) and antisense 5'-GCAGCAGTGGTCGCTCCAGTGT-3' (SEQ ID NO:14). In addition, PCR reactions using the three pairs of primers located at the 5'-end, the middle region and the 3'-end (SEQ ID NOs:5-10) showed that these two clones contained all the coding sequence information, indicating that it contained the entire uromodulin gene (Fig. 6).

Identification of the Uromodulin Gene in Multiple Animal Species

An analysis of the conservation of the uromodulin gene sequence in other animal species is shown in Figs. 7A and 7B. The genomic DNA of human, monkey, rat, mouse, dog, cow, rabbit, chicken and yeast were digested with EcoRI restriction enzyme and hybridized with the uromodulin middle region probe described above, using the same Southern blot hybridization conditions used above for screening the mouse kidney cDNA library. The results of the Southern blot hybridization shown in Fig. 7B show that the uromodulin gene is conserved in mammals and is present as a single copy in human, monkey, rat, mouse, dog, cow and rabbit. Pennica et al (1987) and Yu et al (1994) reported that the gene structure (exons and introns) of

human, bovine and rat uromodulin are highly conserved (Fig. 8).

Identification of Gene Fragments Containing the Mouse Uromodulin Gene Promoter

Southern blotting was performed to identify DNA fragments containing the uromodulin promoter sequence. This approach is based on the differential reactivity of DNA restriction fragments of BAC clone 1 DNA with three different uromodulin probes located in the 5'-end, middle region, and 3'-end of the uromodulin cDNA. Thus, BAC plasmid clone 1 was digested with the restriction enzymes NotI, BamHI, HindIII, PstI, EcoRI, ApaI, NcoI, SacI, XhoI and KpnI. After agarose gel electrophoresis, DNA fragments were transferred onto nylon membrane, UV-crosslinked and hybridized with the 5'-end, middle region, and 3'-end cDNA probes. A 6.9 kb PstI DNA fragment (Fig. 9, lane 4), an 8.3 kb ApaI DNA fragment (Fig. 9, lane 6), and an 8.5 kb SacI DNA fragment (Fig. 9, lane 8) reacted with only the 5'-end probe, but not with middle region probe or the 3'-end probe. This strongly indicates that these three DNA fragments contain portions of the 5'-end of the uromodulin coding sequence and, more importantly, a large fragment of the 5'-upstream region of the mouse uromodulin gene. In contrast, a 9 kb KpnI fragment reacted with all three probes (Fig. 9, lane 10), indicating that this fragment contains all the coding sequences for mouse uromodulin. Finally, a 10 kb EcoRI fragment reacted only with the 3'-probe (Fig. 9, lane 7), indicating that this fragment contains the

3'-end of the coding region and the non-coding region. The identification of DNA fragments containing the entire mouse uromodulin gene, particularly the 5'- upstream sequence facilitates the cloning of the uromodulin gene promoter.

Sequencing of Mouse Uromodulin Promoter

The 8.3 kb ApaI DNA fragment was used for further promoter analysis. A genomic walking method was employed to sequence the entire mouse uromodulin promoter from both 5'- and 3'-ends by sequentially walking the sequence and synthesizing the new primers based on newly obtained sequences. Sequences were determined by the dideoxynucleotide chain termination method of Sanger et al (1977) on an automatic DNA sequencer. Listed below are sense- and anti-sense primers used for the sequencing purposes.

Sense Primers

S1: 5'-TGTCCTATGTGACTCCAGCT-3' (SEQ ID NO:15)

S2: 5'-TCTCCTCAGCTCTCCTGGTC-3' (SEQ ID NO:16)

S3: 5'-TCCTGCCACCACCATGACCA-3' (SEQ ID NO:17)

S4: 5'-AAGCACCGGTGTGCTTGTAT-3' (SEQ ID NO:18)

S5: 5'-ATGGGGCTGCTGAGACTAAG-3' (SEQ ID NO:19)

Anti-sense Primers

AS1: 5'-AAGTCAGACTGTGTTAGGAT-3' (SEQ ID NO:20)

AS2: 5'-ATTGACTGAGCAGGAAGCAT-3' (SEQ ID NO:21)

AS3: 5'-ATTTTATAACCTCCCTCTAG-3' (SEQ ID NO:22)

AS4: 5'-ATGCATTCCAGTCTCAGTGC-3' (SEQ ID NO:23)

AS5: 5'-TGGGGAGAGGACAAAGCCTTG-3' (SEQ ID NO:24)

AS6: 5'-TGACGTGCCAACTCCACTGA-3' (SEQ ID NO:25)

AS7: 5'-AGGACCTGTAGGGTAAGAAA-3' (SEQ ID NO:26)

AS8: 5'-TCTGGCTGTGGGCTCTATAT-3' (SEQ ID NO:27)

Analysis of the Mouse Uromodulin Promoter

The 9,345 bp nucleotide sequence of the promoter region and the genomic coding region including exon 3 of the mouse uromodulin gene is shown in Fig. 10. These results (1) establish the authenticity of the isolated uromodulin clone, (2) indicate that a 7 kb uromodulin promoter has been obtained which is more than adequate to be used in the urine-based transgenic bioreactor system. This mouse promoter can be used in other mammalian species, such as farm animals, to drive the kidney-specific expression of any heterologous gene.

Subcloning of Mouse Uromodulin Promoter

Having identified the mouse uromodulin promoter region, this region can be subcloned for further amplification, and for constructing transgenes. Since the clone containing the uromodulin promoter region is at least 70 kb in size, restriction digestion of each of this clone gives rise to multiple bands. Although the relative sizes of uromodulin promoter-containing bands can be determined by Southern blotting using the 5'-end probe, this does not allow for pinpointing a specific band for subcloning, as most bands are not well-resolved. To circumvent this problem, a dot-blot approach by gel-purifying each individual band in the close vicinity of the area where Southern blot hybridization revealed a positive band will be taken. DNA in each band will

be eluted using a QIAEX column (QIAGEN), and then blotted onto nylon membrane, UV-crosslinked and hybridized with a uromodulin 5'-probe. The bands reacting with the probe will then be subjected to subcloning.

The plasmid pBluescript (Stratagene, LaJolla, CA), which was used as the cloning vector, is to be restriction-digested using PstI, ApaI and SacI, respectively, phosphatase-treated, and the linearized pBluescript cloning vectors will be mixed with the correspondingly digested inserts, ligation buffer, T4 DNA ligase, and incubated at 16°C for 16 hours. Half of this ligation mixture will be used to transform $CaCl_2$-prepared competent JM109 bacterial cells and then screened using small-scale plasmid preparations, which are carried out using mini-prep columns (Promega) and then restriction-digested to release the inserts. Through these procedures, the DNA fragments containing mouse uromodulin promoter are to be subcloned.

Detailed Restriction Mapping of Mouse Uromodulin Promoter

Restriction mapping of the 5'-flanking sequence of uromodulin, an important step for determining the restriction fragments for constructing transgenes has been performed. Although the detailed restriction map is not shown here, such a restriction map can be generated quite readily using any of the numerous publicly or commercially available DNA analysis software programs. A schematic presentation of the mouse uromodulin promoter with several restriction sites denoted is shown in Fig. 11.

EXAMPLE 2: ISOLATION OF GOAT UROMODULIN GENE PROMOTER

Isolation of Goat Uromodulin cDNA

The goat uromodulin cDNA was isolated using reverse transcriptase/polymerase chain reaction (RT-PCR) approach (Wu, et al., 1993). Briefly, a sense and an antisense primer were synthesized based on the mouse uromodulin gene sequence that was isolated in the laboratory of the present inventors. The sequences of these two primers are:

5'-GACTGAGTACTGGCGCAGCACAG-3' (SEQ ID NO:28) and

5'-GATTGCACTCAGGGGGCTCTGT-3' (SEQ ID NO:29). Total RNA was isolated from goat kidneys using the guanidine isothiocyanate method, reverse-transcribed using AMV reverse transcriptase, and the second strand of cDNA was synthesized using DNA polymerase I. PCR amplification was performed using total kidney cDNAs as templates and the two mouse uromodulin as primers, in the presence of dNTP, Taq polymerase, and PCR buffer. The PCR reaction was performed for 35 cycles of denaturation at 94°C, annealing at 55°C and extension at 72°C and the resulting PCR products were resolved by agarose gel. The products having the predicted size were subcloned into the TA cloning vector (Invitrogen, Carlsbad, CA) and sequenced.

RT-PCR of goat kidney-derived mRNAs, using the pair of primers derived from mouse uromodulin, yielded a single, approximately 300 bp product upon agarose gel electrophoresis. The PCR product was subcloned and sequenced. A Blast search of Genbank of the PCR product sequence (SEQ ID NO:2; Fig.12) showed that the top four hits were uromodulin sequences from several species. Thus, the sequence of the PCR product shared

a 96% identity (287 bp/297 bp) with bovine uromodulin, 90% identity (218/241) with human uromodulin, a 78% identity (239/304) with rat uromodulin, and an 80% identity in a shorter stretch (125/156) with mouse uromodulin. The high degree of sequence identity of the PCR product with known uromodulin sequences firmly established that the product is a partial goat uromodulin cDNA.

Isolation of Goat Uromodulin Genomic DNA By Genomic Walking, Cloning and Sequencing

A genomic walking approach was employed to isolate the goat uromodulin gene using specific sequence information obtained from goat uromodulin cDNA. Genomic DNA was isolated from goat kidneys and used as templates for PCR-based genomic walking (Clontech, Palo Alto, CA). The genomic DNA was digested using five restriction enzymes (DraI, ScaI, EcoRV, PvuII, StuI), each of which created a blunt end in the genomic DNAs. The ends were ligated with adaptors. PCR was then performed using the ligated DNA library as templates, and two independent anti-sense primers synthesized based on the newly obtained uromodulin cDNA sequence as well as a sense primer located on the adaptor. The sequences for the two anti-sense primers are 5'-GTACCAGCCGCCCAGACTGACATCACAG-3' (SEQ ID NO:30; primer AS14), and 5'-CAGGTTGTACACGTAGTAGCCGCCGGCA-3' (SEQ ID NO:31; primer AS17). The PCR was performed for 1 cycle of denaturation at 99°C for 5 sec, annealing and extension at 68°C for 4 min., followed by 7 cycles of denaturation at 94°C for 2 sec, annealing and extension at 68°C for 4 min., followed by 32 cycles of denaturation at 94°C for 2 sec,

annealing and extension at 63°C for 4 min., and followed by 1 cycle at 63°C for 4 min. After the first round of PCR, the products were used as templates and subjected to a second round of PCR amplification using two new, nesting sense and anti-sense primers. The specific products were subcloned into the TA cloning vector and the identity of the goat uromodulin gene was confirmed by DNA sequencing of both ends of the product.

Based on the newly identified goat uromodulin cDNA, the two above anti-sense primers were designed for genomic walking using goat genomic DNA to identify DNA sequences that are located in the upstream region. After the first and second rounds of PCR and nesting PCR amplifications, a 1.5 kb, single PCR product was obtained. Subcloning and sequencing of this product revealed that its 3'-end shares 94% identity (494/522) with bovine uromodulin cDNA sequence, thus confirming that the PCR product is a portion of the goat uromodulin gene. The 5'-sequence did not share any significant homology with any of the known uromodulin cDNA sequences and therefore most likely represents intron sequences. Based on the gene structure of mouse uromodulin and the relative length (1.5 kb) of the PCR product, this 5'-sequence is most likely located in intron 1. The nucleotide sequences of intron 1 (SEQ ID NO:3) and exon 3 (SEQ ID NO:4) of the goat uromodulin gene are shown in Figs. 13A and 13B, respectively.

Isolation of Goat Uromodulin Promoter by Secondary Genomic Walking

For the isolation of goat uromodulin promoter, the 5'-end of the genomic clone that was isolated from the first round of genomic walking was used to design new antisense "walking primers" located in intron 1. The five primers are:

5'-AAGATTTACCAGCCCGGGCCGTCGACC-3' (SEQ ID NO:32; AS1)

5'-AATAAAGTGCCAGGGCAGGGGGGCTTA-3' (SEQ ID NO:33; AS2)

5'-CTTGTGTGGTTGAGTGTGTTCTTGACC-3' (SEQ ID NO:34; AS3)

5'-TGTGAAAGGGGATGTCTTTGGGTACCA-3' (SEQ ID NO:35; AS4)

5'-ACAGCAATGTGCAACCCAATGGAAGGG-3' (SEQ ID NO:36; AS5).

Fresh goat genomic DNA as template was digested by the five blunt-ending restriction enzymes (see above) and subjected to PCR walking using these five anti-sense primers and the aforementioned conditions.

PCR and nesting PCR yielded a highly specific, 1.0 kb product in three independent primer combinations. A further round of genomic walking resulted in a 1.6 kb fragment which was subcloned as smaller fragments. Subcloning and DNA sequencing of the subcloned fragments provided the 1.6 kb goat uromodulin promoter sequence of SEQ ID NO:37 and its structural features as shown in Figs. 14A and 14B. A computer comparison/alignment of the nucleotide sequences of the mouse and goat uromodulin promoter regions is presented in Figs. 15A and 15B.

EXAMPLE 3: CONSTRUCTION OF KIDNEY-BASED BIOREACTOR SYSTEM

Construction of Chimeric Genes

To test the tissue-specificity of the uromodulin gene promoter and its utility in a kidney-based bioreactor system, a chimeric gene containing a uromodulin promoter and a gene encoding a pharmaceutically-important protein is to be constructed. For this purpose, human growth hormone (hGH), whose expression has been recently assessed in a uroplakin II-based, bladder bioreactor system (Kerr et al, 1998) will be tested first. A potential limitation has been recognized with the bladder bioreactor system in that it produced relatively low amounts of hGH. Such a potential limitation may possibly be associated with the less than optimal secretory activity of the urothelium. Since uromodulin is normally synthesized in the ascending limb of Henle's loop and the distal tubules where active secretion takes place, the present inventors expect that there will be an active secretion of synthesized hGH into the urine of mice, resulting in high protein yield. The presence of this uromodulin/hGH gene in transgenic mice will allow a comparison of the efficiency between the kidney-based and the bladder-based reactor systems.

An 8.8 kb genomic fragment containing the 5'-upstream region of the mouse uromodulin gene was used as a template for PCR amplification to yield a 3.0 kb uromodulin promoter fragment. PCR sense (SEQ ID NO:53) and antisense (SEQ ID NO:54) primers were designed so that their ends included an ApaI enzyme cleavage site to facilitate cloning. The 3.0 kb PCR fragment was subcloned into the ApaI site of

the pBluescript vector. A 2.1 kb genomic fragment of human growth hormone gene (Genbank accession number M13438 for complete coding sequence of hGH) containing the entire coding sequences was excised from phGH-N vector (obtained from Brian M. Shewchuk, Department of Genetics, University of Pennsylvania, Philadelphia, PA), gel-purified and subcloned into the BamHI site of the above-mentioned pBluescript vector so that human growth gene is positioned downstream of the mouse uromodulin promoter (Fig. 16). The correct orientation of the chimeric gene was verified by restriction digestion and DNA sequencing. The uromodulin-hGH chimeric gene was retrieved en bloc by restriction digestion using KpnI and XbaI. The 5.1 kb fragment was resolved by agarose gel electrophoresis, electroeluted and dialyzed extensively against Tris-EDTA buffer. The purified chimeric gene was then microinjected into the fertilized eggs of FVB/N inbred mice and implanted into the uteri of pseudopregnant mice as previously described by Brinster et al.(1981).

Southern blotting was employed to identify transgenic mice harboring the chimeric uromodulin-hGH gene. DNA was extracted from mouse tail using proteinase K digestion and NaCl precipitation. The DNA was digested with BglII, electrophoresed and transferred onto nylon membrane and hybridized with a 500 bp probe located at the 3'-end of the uromodulin promoter (Fig. 17). Out of 42 live-born animals, three carried the transgene as evidenced by the appearance of a 5.1 kb transgene fragment in the Southern blot of mouse tail DNA (Fig. 17). In Fig. 17, lanes 1 and 5 are non-transgenic

control mice showing the endogenous fragment (Endo) of uromodulin gene, transgene fragments (Trans), in mouse 2 (15 kb), mouse 3 (9 kb) and mouse 4 (5.5 kb and 4.9 kb). These different fragment sizes may reflect the particular transgene orientation and the chromosomal site of transgene integration.

Expression of hGH in Mouse Kidney

The expression of hGH in transgenic mouse kidney is to be assessed at both the mRNA and protein levels. RT-PCR will be performed to determine the expression of mRNA using primers specific for hGH. Total RNAs will be extracted from transgenic mouse kidneys and from control tissues, including rat liver, skin, intestine, stomach, brain, skeletal muscle, thymus, thyroid gland, bladder, lungs, heart, pancreas, spleen, prostate, seminal vesicles, uterus and ovaries. The total RNAs are to be reverse-transcribed, PCR amplified and analyzed by agarose gel electrophoresis. The results will reveal whether hGH is expressed in kidney-dependent fashion. To determine whether hGH was synthesized in the ascending limb of Henle's loop and the distal tubules of the kidney, immunofluorescent staining of the kidney using anti-hGH antibody will be performed. Frozen kidney sections are to be stained using an indirect immunofluorescent method (Wu et al, 1993).

The laboratory of the present inventors have now performed radioimmunoassays (RIA) to determine the level of hGH in the urine and the serum of the transgenic mice. Urine samples were collected from transgenic mice by gently

massaging the lower abdomen of the mice. Fresh samples were subjected to RIA without further processing. An RIA assay kit from Nichols Institute Diagnostics (San Juan Capistrano, CA) was used and ^{125}I-labeled hGH was obtained from Dupont NEN, (Billerica, MA). The standard curve was prepared by plotting the corrected CPM of each standard level against the standard concentration of hGH. The value of the urinary hGH concentration was obtained by referencing the CPM reading of the urine samples. For serum hGH measurement, whole blood was obtained from mouse tails and serum was isolated and subjected to RIA as described above.

Figure 18 shows the results from urine samples of two transgene-negative (-) and three transgene-positive mice (NOs. 1, 7, and 8) subjected to RIA. Human growth hormone was detected in transgenic mice Nos. 7 and 8, but not in transgenic mouse No. 1 or in the transgene-negative mice. The concentration of hGH in the two positive Nos. 7 and 8 mice were 20 and 22 ng/ml, respectively.

The urine and serum concentration of hGH in transgenic mice were also compared by RIA. Figure 19 shows the results of RIA performed on serum and urine samples from transgenic mice. The high concentration of hGH in transgenic mouse No. 8 (15 ng/ml) indicates leakage into the blood of hGH synthesized by kidney epithelial cells.

In order to assess the sensitivity of the RIA assay for hGH, known amounts of hGH were added into the same volume of urine sample from non-transgenic (normal) mice and then subjected to RIA. From Fig. 20, the recovery of hGH by this

assay is observed to be nearly 100% for hGH amounting to 10 ng/ml with the recovery being observed to decrease to 90% and 60%, respectively, when the hGH concentration increases to 20 to 50 ng/ml.

Having now fully described this invention, it will be appreciated that by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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WHAT IS CLAIMED IS:

1. An isolated DNA molecule, comprising a kidney-specific promoter operably linked to a heterologous DNA sequence encoding a heterologous polypeptide containing a non-native apical surface membrane targeting sequence, wherein said kidney-specific promoter is capable of driving the expression of said heterologous polypeptide *in vivo* in the kidneys to produce a recombinant biologically active polypeptide in the urine.

2. An isolated DNA molecule according to claim 1, wherein said kidney-specific promoter is a uromodulin promoter.

3. An isolated DNA according to claim 2, wherein said uromodulin promoter is a goat uromodulin promoter.

4. An isolated DNA according to claim 3, wherein said goat uromodulin promoter has the nucleotide sequence of SEQ ID NO:37, or a fragment thereof capable of directing kidney-specific expression.

5. An isolated DNA according to claim 2, wherein said uromodulin promoter is the mouse uromodulin promoter.

6. An isolated DNA molecule according to claim 5, wherein said mouse uromodulin promoter has the nucleotide sequence of SEQ ID NO:1, or a fragment thereof capable of directing kidney-specific expression.

7. An isolated DNA molecule according to claim 1, wherein said non-native apical surface membrane targeting

sequence is a C-terminal glycosyl phosphatidylinositol (GPI) signal sequence.

8. An isolated DNA molecule according to claim 1, wherein said apical surface membrane targeting sequence is one or more non-native sites for glycosylation at predicted β-turns of said heterologous polypeptide.

9. An isolated DNA molecule according to claim 8, wherein said one or more non-native sites for glycosylation are sites for Asn-linked glycosylation.

10. An isolated DNA molecule according to claim 8, wherein said one or more non-native sites for glycosylation are sites for O-glycosylation.

11. An isolated DNA according to claim 1, further comprising a secretion signal sequence operably linked to said heterologous DNA sequence.

12. An isolated DNA molecule according to claim 1, wherein said heterologous polypeptide is a fusion polypeptide.

13. An isolated DNA molecule according to claim 9, wherein said fusion polypeptide is a fusion between a heterologous polypeptide of interest and uromodulin via a chemically or enzymatically cleavable linker, said uromodulin having a GPI signal sequence at its C-terminus.

14. An isolated DNA molecule according to claim 13, wherein said linker is a protease-sensitive linker.

15. An isolated DNA molecule according to claim 1, further comprising a DNA sequence encoding phosphatidylinositol-specific phospholipase C (PIPLC), wherein said DNA sequence is disposed 3' of said heterologous DNA

sequence and is operably linked to said kidney-specific promoter, whereby said kidney-specific promoter is capable of driving the expression of said DNA sequence encoding PIPLC.

16. An isolated DNA molecule according to claim 1, wherein any basolateral surface membrane targeting signals native to said heterologous polypeptide is inactivated or deleted.

17. An isolated DNA molecule according to claim 1, further comprising a self-replicable vector.

18. A host cell transformed with the DNA molecule of claim 1.

19. A method for producing a recombinant biologically active polypeptide, comprising:

introducing the isolated DNA molecule of claim 1 into a fertilized embryo of a non-human mammal to generate a transgenic non-human mammal which expresses and secretes the heterologous polypeptide into the urine of the transgenic non-human mammal as a recombinant biologically active polypeptide;

collecting urine from the transgenic non-human mammal; and

recovering the secreted polypeptide to produce a recombinant biologically active polypeptide.

20. A method according to claim 19, wherein said introducing step comprises injecting the isolated DNA molecule into a pronucleus of a fertilized embryo.

21. A method according to claim 19, wherein the isolated DNA comprises a uromodulin promoter operably linked to a heterologous DNA sequence.

22. A method according to claim 21, wherein the uromodulin promoter is a mouse, goat, bovine or rat uromodulin promoter.

23. A method according to claim 21, wherein the uromodulin promoter is a goat uromodulin promoter.

24. A method according to claim 19, wherein said non-human mammal is a goat, cow, sheep, pig or horse.

25. A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant construct corresponding to the DNA molecule of claim 1, said DNA molecule having been introduced into said mammal, or an ancestor of said mammal, at an embryonic stage, and wherein said mammal produces recoverable amounts of a recombinant biologically active polypeptide in its urine.

26. A transgenic non-human mammal according to claim 25 which is a transgenic goat, cow, sheep, pig or horse.

27. A transgenic non-human mammal according to claim 25, which is a transgenic goat.

28. A transgenic non-human mammal according to claim 25, in which all germ cells and somatic cells further contains a recombinant construct comprising a kidney-specific promoter operably linked to a DNA sequence encoding PIPLC, wherein said kidney-specific promoter expresses PIPLC in the kidneys of said transgenic mammal.

29. An isolated DNA molecule, comprising a kidney-specific promoter operably linked to a heterologous DNA sequence encoding a heterologous polypeptide in which

basolateral surface membrane targeting signals are inactivated or deleted.

30. An isolated DNA molecule according to claim 29, wherein said kidney-specific promoter is a uromodulin promoter.

31. An isolated DNA according to claim 30, wherein said uromodulin promoter is a goat uromodulin promoter.

32. An isolated DNA according to claim 31, wherein said goat uromodulin promoter has the nucleotide sequence of SEQ ID NO:37, or a fragment thereof capable of directing kidney-specific expression.

33. An isolated DNA according to claim 30, wherein said uromodulin promoter is the mouse uromodulin promoter.

34. An isolated DNA molecule according to claim 33, wherein said mouse uromodulin promoter has the nucleotide sequence of SEQ ID NO:1, or a fragment thereof capable of directing kidney-specific expression.

35. An isolated DNA according to claim 29, further comprising a secretion signal sequence operably linked to said heterologous DNA sequence.

36. An isolated DNA molecule according to claim 29, further comprising a self-replicable vector.

37. A host cell transformed with the DNA molecule of claim 29.

38. A method for producing a recombinant biologically active polypeptide, comprising:

introducing the isolated DNA molecule of claim 29, into a fertilized embryo of a non-human mammal to generate a

transgenic non-human mammal which expresses and secretes the heterologous polypeptide into the urine of the transgenic non-human mammal as a recombinant biologically active polypeptide;

collecting urine from the transgenic non-human mammal; and

recovering the secreted polypeptide to produce a recombinant biologically active polypeptide.

39. A method according to claim 38, wherein said introducing step comprises injecting the isolated DNA molecule into a pronucleus of a fertilized embryo.

40. A method according to claim 38, wherein the isolated DNA comprises a uromodulin promoter operably linked to a heterologous DNA sequence.

41. A method according to claim 40, wherein the uromodulin promoter is a mouse, goat, bovine or rat uromodulin promoter.

42. A method according to claim 40, wherein the uromodulin promoter is a goat uromodulin promoter.

43. A method according to claim 38, wherein said non-human mammal is a goat, cow, sheep, pig or horse.

44. A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant construct corresponding to the DNA molecule of claim 29, said DNA molecule having been introduced into said mammal, or an ancestor of said mammal, at an embryonic stage, and wherein said mammal produces recoverable amounts of a recombinant biologically active polypeptide in its urine.

45. A transgenic non-human mammal according to claim 44, which is a transgenic goat, cow, sheep, pig or horse.

46. A transgenic non-human mammal according to claim 44, which is a transgenic goat.

ABSTRACT OF THE DISCLOSURE

The invention relates to recombinant DNA constructs, a method for producing a recombinant biologically active protein *in vivo* in the urine of a non-human mammal using a kidney-specific promoter, such as the uromodulin promoter, and the transgenic non-human mammals that serve as urine-based bioreactors for protein production.

F:\,N\nyum\wu43c\specification 2.wpd

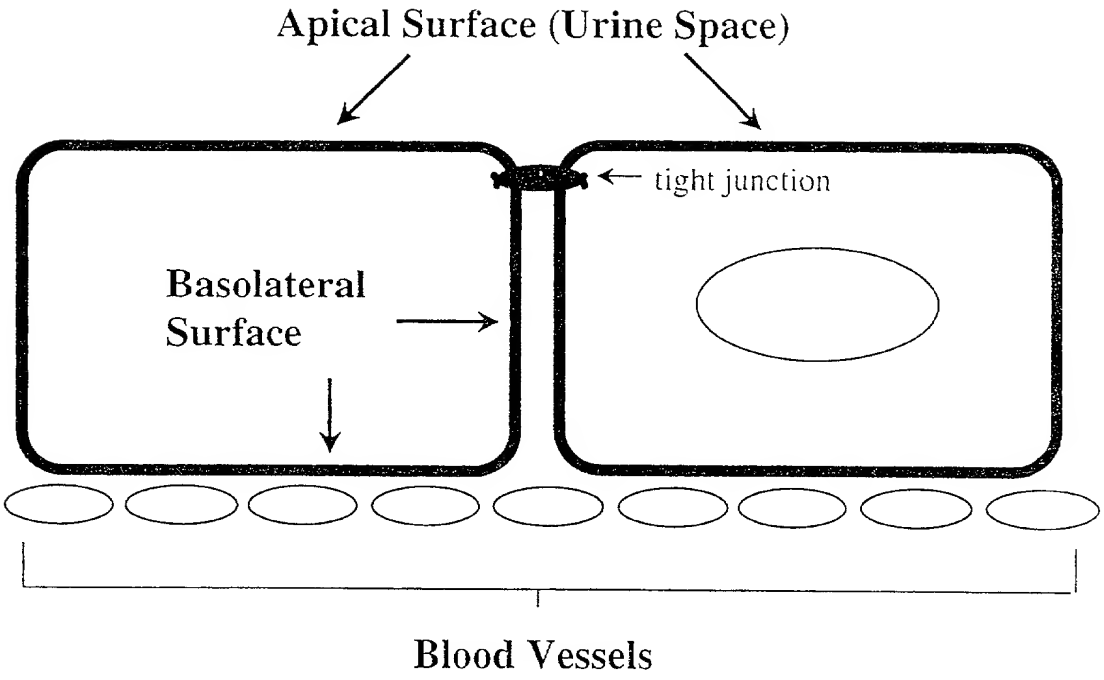

Apical Surface (Urine Space)
tight junction
Basolateral Surface
Blood Vessels

FIG.1

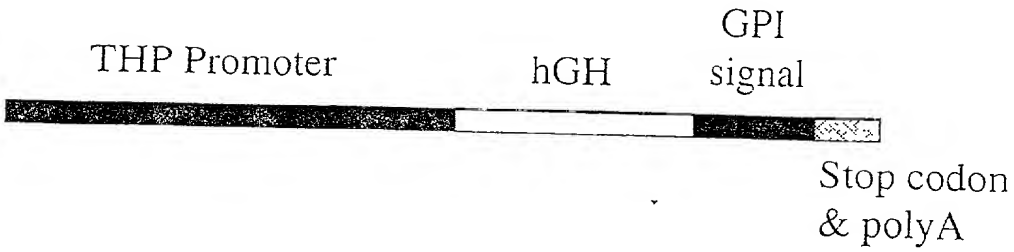

THP Promoter
hGH
GPI signal
Stop codon & polyA

FIG.2

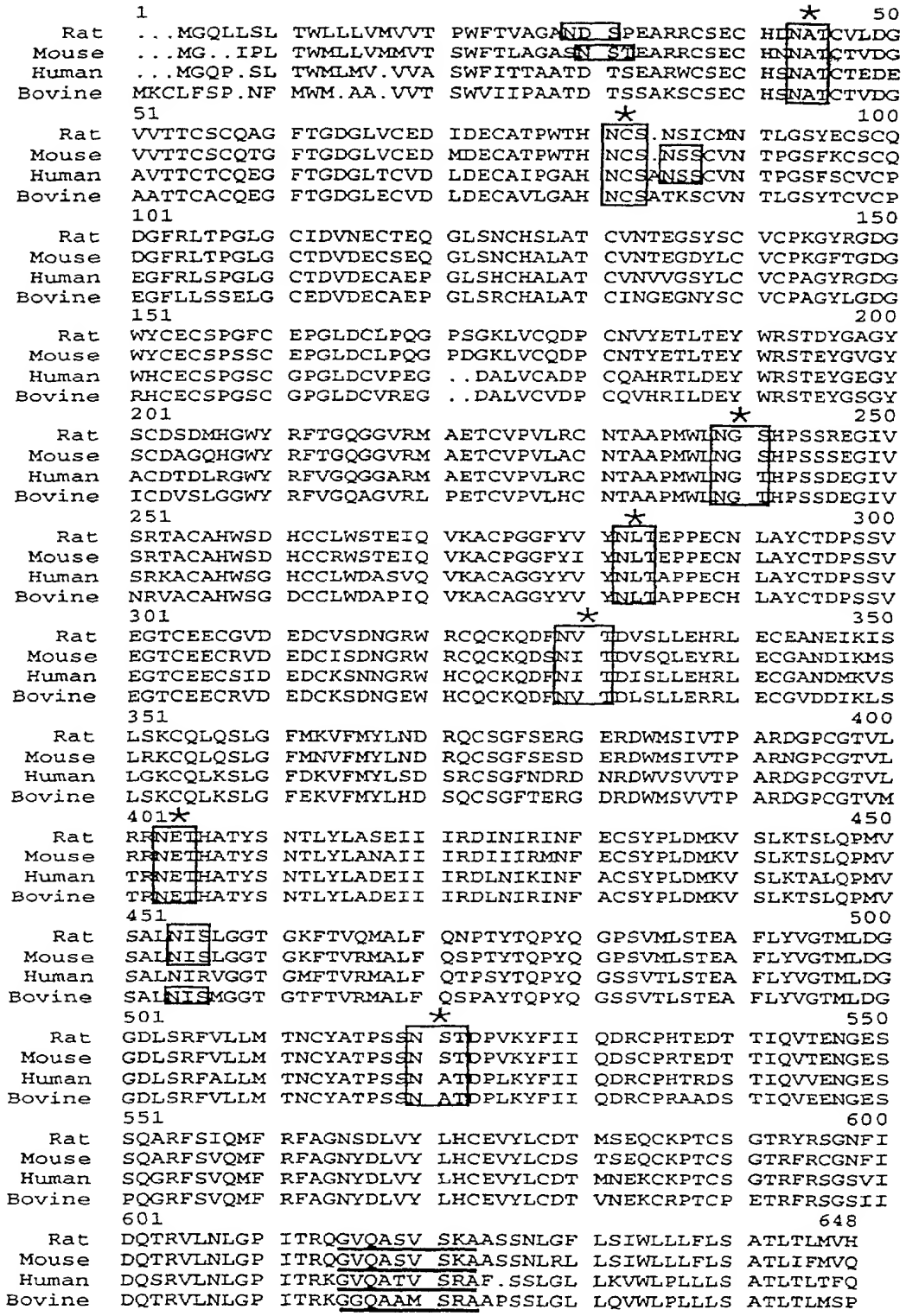

1 50
Rat ...MGQLLSL TWLLLVMVVT PWFTVAGAND SPEARRCSEC HDNATCVLDG
Mouse ...MG..IPL TWMLLVMMVT SWFTLAGASN STEARRCSEC HNNATCTVDG
Human ...MGQP.SL TWMLMV.VVA SWFITTAATD TSEARWCSEC HSNATCTEDE
Bovine MKCLFSP.NF MWM.AA.VVT SWVIIPAATD TSSAKSCSEC HSNATCTVDG
51 100
Rat VVTTCSCQAG FTGDGLVCED IDECATPWTH NCS.NSICMN TLGSYECSCQ
Mouse VVTTCSCQTG FTGDGLVCED MDECATPWTH NCS.NSSCVN TPGSFKCSCQ
Human AVTTCTCQEG FTGDGLTCVD LDECAIPGAH NCSANSSCVN TPGSFSCVCP
Bovine AATTCACQEG FTGDGLECVD LDECAVLGAH NCSATKSCVN TLGSYTCVCP
101 150
Rat DGFRLTPGLG CIDVNECTEQ GLSNCHSLAT CVNTEGSYSC VCPKGYRGDG
Mouse DGFRLTPGLG CTDVDECSEQ GLSNCHALAT CVNTEGDYLC VCPKGFTGDG
Human EGFRLSPGLG CTDVDECAEP GLSHCHALAT CVNVVGSYLC VCPAGYRGDG
Bovine EGFLLSSELG CEDVDECAEP GLSRCHALAT CINGEGNYSC VCPAGYLGDG
151 200
Rat WYCECSPGFC EPGLDCLPQG PSGKLVCQDP CNVYETLTEY WRSTDYGAGY
Mouse WYCECSPSSC EPGLDCLPQG PDGKLVCQDP CNTYETLTEY WRSTEYGVGY
Human WHCECSPGSC GPGLDCVPEG ..DALVCADP CQAHRTLDEY WRSTEYGEGY
Bovine RHCECSPGSC GPGLDCVREG ..DALVCVDP CQVHRILDEY WRSTEYGSGY
201 250
Rat SCDSDMHGWY RFTGQGGVRM AETCVPVLRC NTAAPMWLNG SHPSSREGIV
Mouse SCDAGQHGWY RFTGQGGVRM AETCVPVLAC NTAAPMWLNG SHPSSSEGIV
Human ACDTDLRGWY RFVGQGGARM AETCVPVLRC NTAAPMWLNG THPSSDEGIV
Bovine ICDVSLGGWY RFVGQAGVRL PETCVPVLHC NTAAPMWLNG THPSSDEGIV
251 300
Rat SRTACAHWSD HCCLWSTEIQ VKACPGGFYV YNLTEPPECN LAYCTDPSSV
Mouse SRTACAHWSD HCCRWSTEIQ VKACPGGFYI YNLTEPPECN LAYCTDPSSV
Human SRKACAHWSG HCCLWDASVQ VKACAGGYYV YNLTAPPECH LAYCTDPSSV
Bovine NRVACAHWSG DCCLWDAPIQ VKACAGGYYV YNLTAPPECH LAYCTDPSSV
301 350
Rat EGTCEECGVD EDCVSDNGRW RCQCKQDFNV TDVSLLEHRL ECEANEIKIS
Mouse EGTCEECRVD EDCISDNGRW RCQCKQDSNI TDVSQLEYRL ECGANDIKMS
Human EGTCEECSID EDCKSNNGRW HCQCKQDFNI TDISLLEHRL ECGANDMKVS
Bovine EGTCEECRVD EDCKSDNGEW HCQCKQDFNV TDLSLLERRL ECGVDDIKLS
351 400
Rat LSKCQLQSLG FMKVFMYLND RQCSGFSERG ERDWMSIVTP ARDGPCGTVL
Mouse LRKCQLQSLG FMNVFMYLND RQCSGFSESD ERDWMSIVTP ARNGPCGTVL
Human LGKCQLKSLG FDKVFMYLSD SRCSGFNDRD NRDWVSVVTP ARDGPCGTVL
Bovine LSKCQLKSLG FEKVFMYLHD SQCSGFTERG DRDWMSVVTP ARDGPCGTVM
401 450
Rat RRNETHATYS NTLYLASEII IRDINIRINF ECSYPLDMKV SLKTSLQPMV
Mouse RRNETHATYS NTLYLANAII IRDIIIRMNF ECSYPLDMKV SLKTSLQPMV
Human TRNETHATYS NTLYLADEII IRDLNIKINF ACSYPLDMKV SLKTALQPMV
Bovine TRNETHATYS NTLYLADEII IRDLNIRINF ACSYPLDMKV SLKTSLQPMV
451 500
Rat SALNISLGGT GKFTVQMALF QNPTYTQPYQ GPSVMLSTEA FLYVGTMLDG
Mouse SALNISLGGT GKFTVRMALF QSPTYTQPYQ GPSVMLSTEA FLYVGTMLDG
Human SALNIRVGGT GMFTVRMALF QTPSYTQPYQ GSSVTLSTEA FLYVGTMLDG
Bovine SALNISMGGT GTFTVRMALF QSPAYTQPYQ GSSVTLSTEA FLYVGTMLDG
501 550
Rat GDLSRFVLLM TNCYATPSSN STDPVKYFII QDRCPHTEDT TIQVTENGES
Mouse GDLSRFVLLM TNCYATPSSN STDPVKYFII QDSCPRTEDT TIQVTENGES
Human GDLSRFALLM TNCYATPSSN ATDPLKYFII QDRCPHTRDS TIQVVENGES
Bovine GDLSRFVLLM TNCYATPSSN ATDPLKYFII QDRCPRAADS TIQVEENGES
551 600
Rat SQARFSIQMF RFAGNSDLVY LHCEVYLCDT MSEQCKPTCS GTRYRSGNFI
Mouse SQARFSVQMF RFAGNYDLVY LHCEVYLCDS TSEQCKPTCS GTRFRCGNFI
Human SQGRFSVQMF RFAGNYDLVY LHCEVYLCDT MNEKCKPTCS GTRFRSGSVI
Bovine PQGRFSVQMF RFAGNYDLVY LHCEVYLCDT VNEKCRPTCP ETRFRSGSII
601 648
Rat DQTRVLNLGP ITRQGVQASV SKAASSNLGF LSIWLLLFLS ATLTLMVH
Mouse DQTRVLNLGP ITRQGVQASV SKAASSNLRL LSIWLLLFLS ATLIFMVQ
Human DQSRVLNLGP ITRKGVQATV SRAF.SSLGL LKVWLPLLLS ATLTLTFQ
Bovine DQTRVLNLGP ITRKGGQAAM SRAAPSSLGL LQVWLPLLLS ATLTLMSP

FIG.3

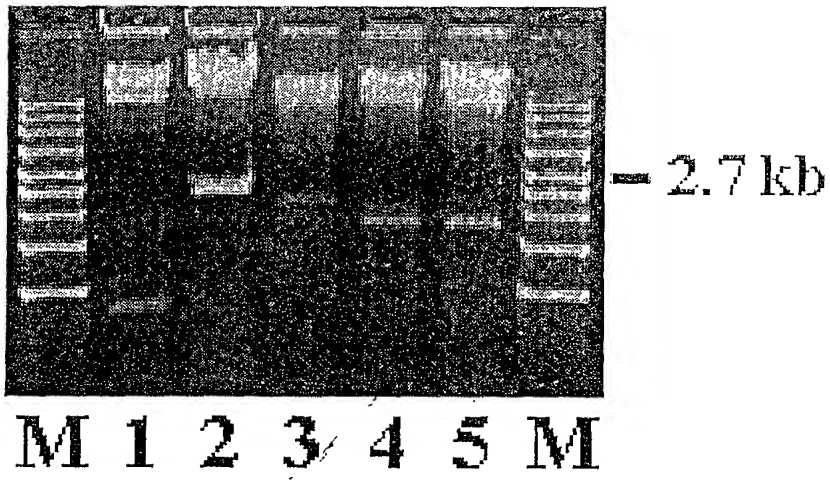


FIG. 4

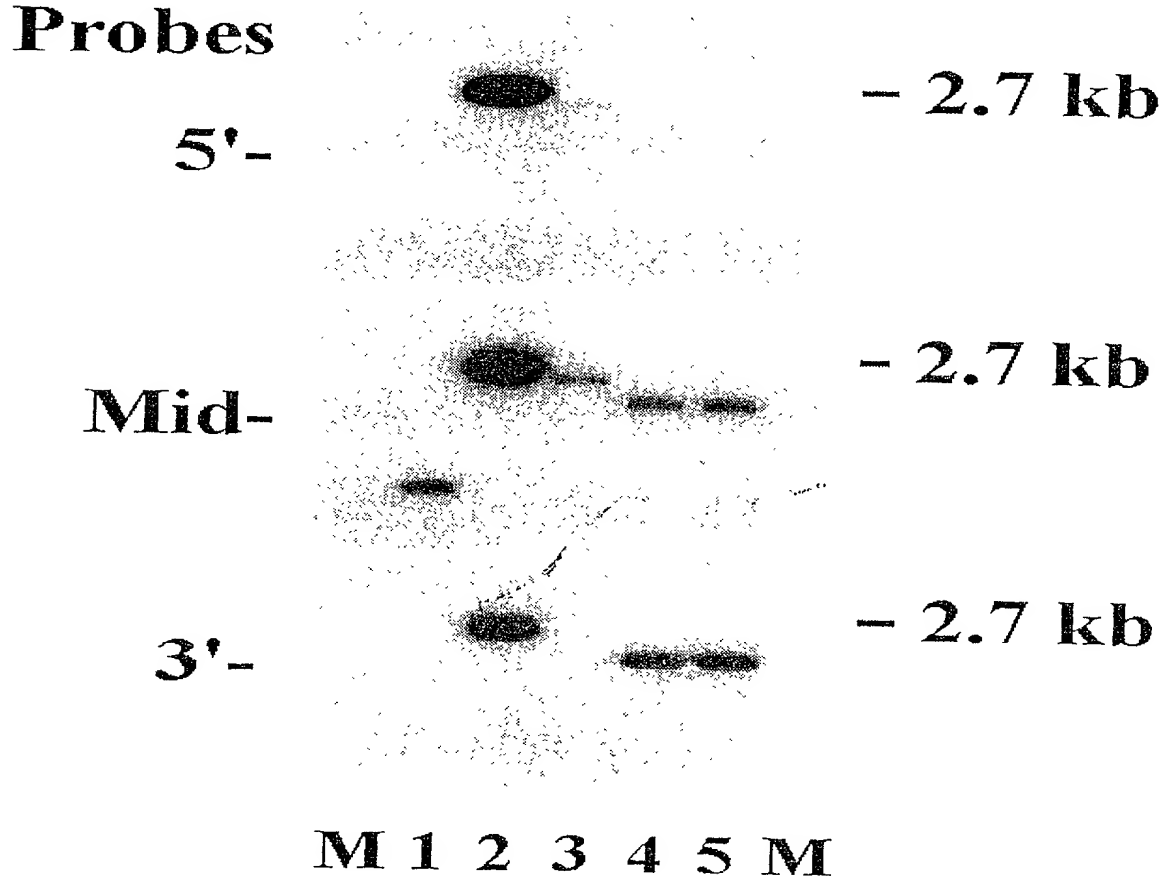


FIG.5

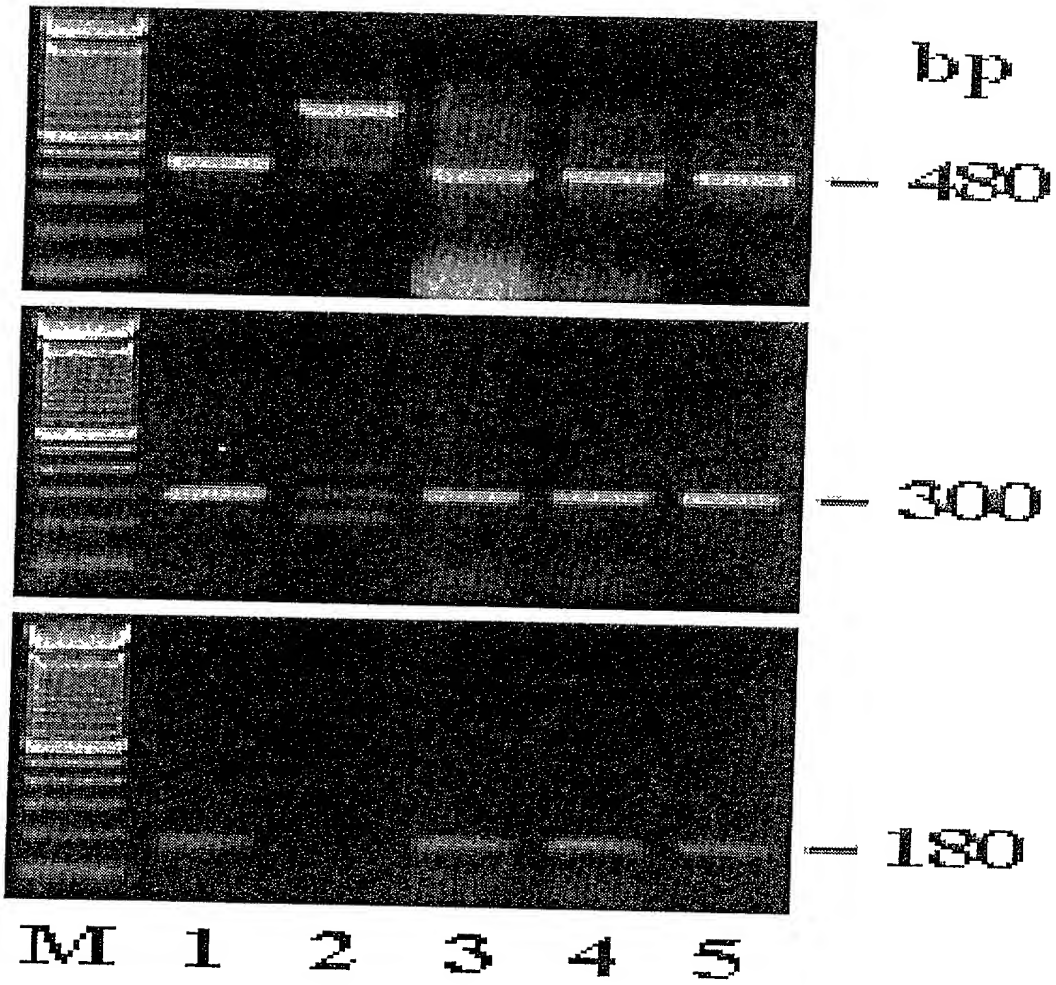


FIG. 6

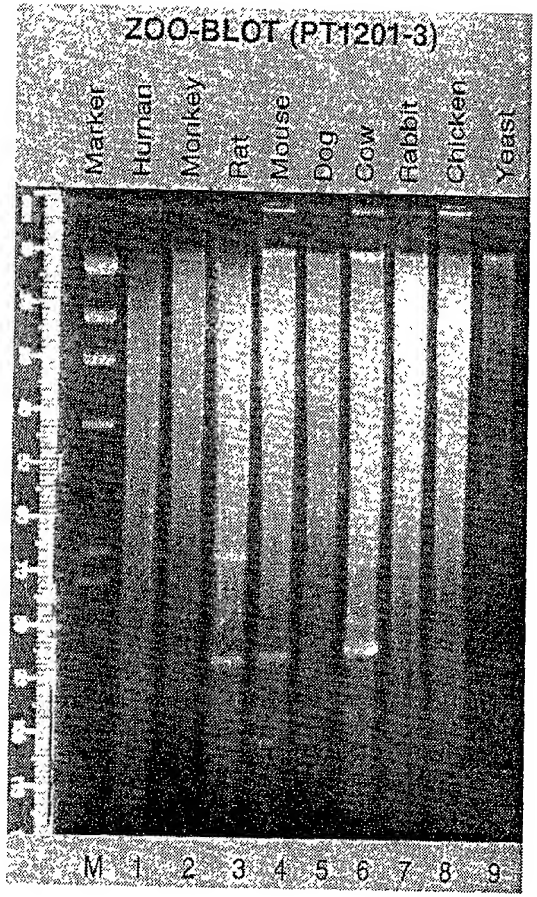

ZOO-BLOT (PT1201-3)
Marker
Human
Monkey
Rat
Mouse
Dog
Cow
Rabbit
Chicken
Yeast
M 1 2 3 4 5 6 7 8 9

FIG. 7A

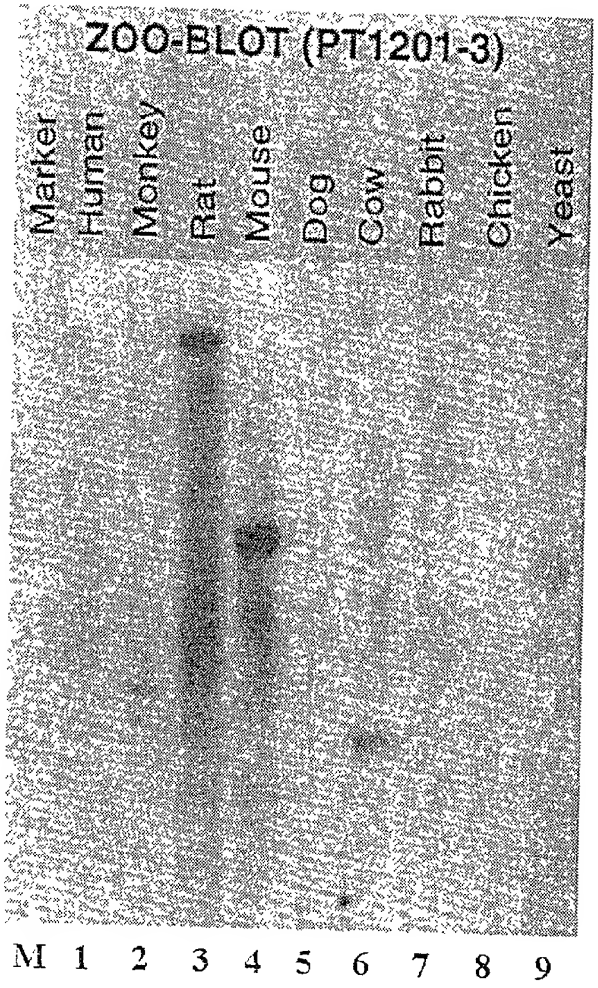

ZOO-BLOT (PT1201-3)
Marker
Human
Monkey
Rat
Mouse
Dog
Cow
Rabbit
Chicken
Yeast
M 1 2 3 4 5 6 7 8 9

FIG. 7B

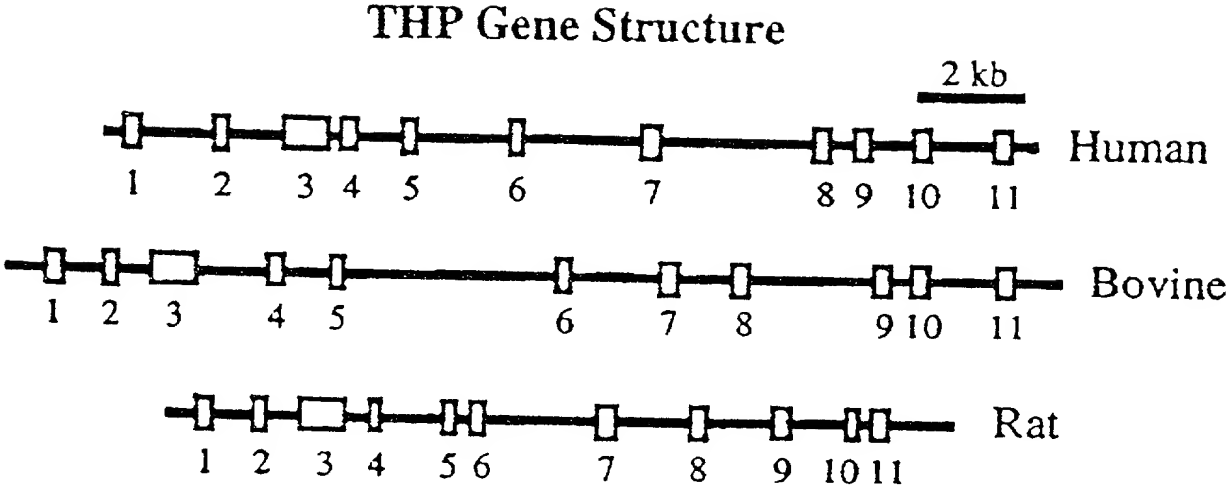

THP Gene Structure
2 kb
Human
1 2 3 4 5 6 7 8 9 10 11
Bovine
1 2 3 4 5 6 7 8 9 10 11
Rat
1 2 3 4 5 6 7 8 9 10 11

FIG. 8

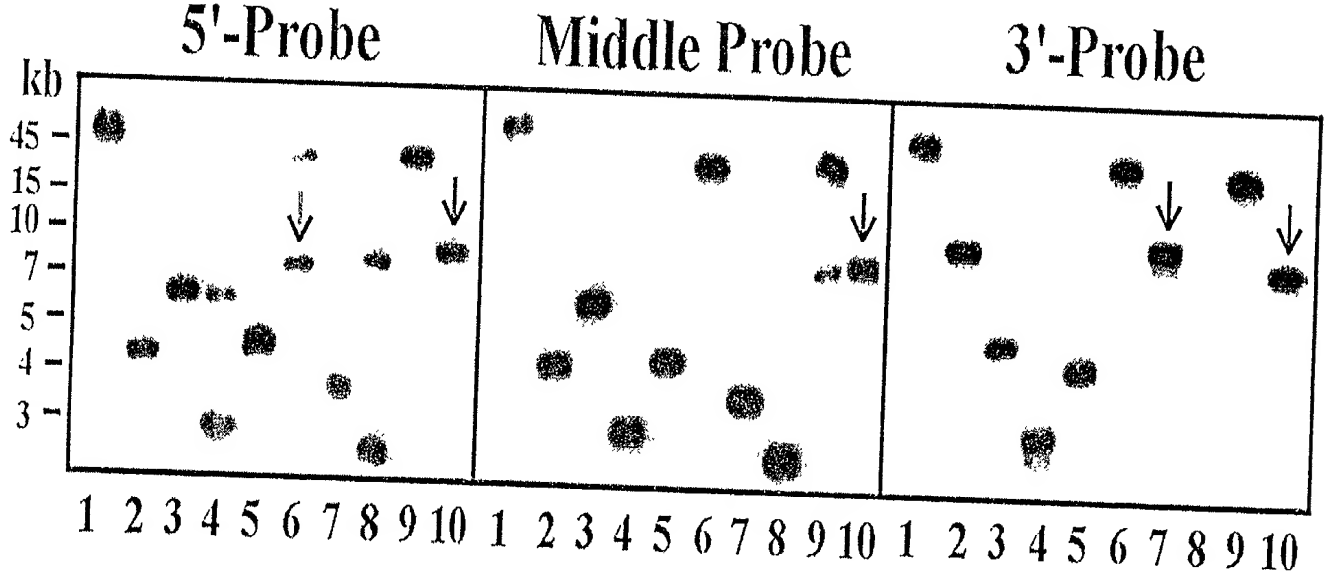


FIG. 9

```
   1  GGGG/GGGCCC TCGGGAGTTT GGCTAAGTCT TGCAAATGAG CTGTGATGAC
      polycloning site of pBS
  51  AGGTTTGCGC CATATGAGAT CCAGTGACAA GCTCATCTCT AGATGTCTGC

 101  ATACCAATAA GTGACCCATC ATTATGCAAT CAGGCCGGAC TCATCCTCTG

 151  TGGCTTTGTC TCTTACTACT GTAAACTTGA TAACCTATAT GATTTTACCC

 201  ATTTCCCCTC CATGGCACTC AACTCTCCTC TTCCTATGTG ACCCTACTTA

 251  TGTCCTATGT GACTCCAGCT GCTTCCTTTG ATGAGAGCCA TCCTGTTCTT
         JP.S3
 301  TCTATGTGAC TCTGCTCACT TCTTCCACGT GACTCCACCA ATCTGTCTAC

 351  ATTGCAGAGT CACTCACAGT TTCTTGAGAG CAGAAGACTC AGAACTGATC

 401  TGTCCTCAAT GTCCTCCCTA CACTTTCTCC TCATAATCCA CATATCTAAA

 451  GCTATAGAGA TAATTTCATG CACTATAGCT TTCAGTACTA TCGTATCTAC

 501  TGTCTCTACC CTGTAACTGG TATCTTCATG ACATCTCGAA TATTTCCAAT

 551  TTCTCTATTG CTGCAAAGTC TTGAGAAGTC TAGTCTTATG GATCTCCTTT

 601  TCTCCTCAGG TCTCCTGGTC TCCACACACC ATTCACACTT CTTGAATATT
        JP.S4
 651  CTTTGAACAT AACAAATTCT CTCCATGGGT TTGTTCCCTC TACCCAAATT

 701  CATGCCTTCA GGATACTTAC TCTGCCCCAT CTTCACTCAT CTCTGCTTTG

 751  GTCATTCAAA TCTCAAATGT AGCCATTTCT AAAAGGCTCT CCAAGAGAAT

 801  AATATTTGAA AGCATTTTGC TATTCTATCA AGTGATCATA CAATGTCTGC

 851  TCCTGCCACC ACCATGACCA TCCCCATGAA TACAGACACT GCCTTCTTAG
        JP.S5
 901  TGTTTGCTGT ATGTGTTCTG TGTGGTACAT TGTAGATAAA TGCTGTAATA

 951  AACATCTGTG GAGCAAATTG AATCATCAGA TAGCACCCTC TCTCTGAGAG

1001  GCATGATCTC ATGGTTATCC CCAAAGCATG AGGTAAGGAC ATTATCCCAG

1051  GTCCATGCTG GTTTCCGTAT TGATTGTTTC TAACACAAAC TTAATAGATT

1101  AAAACAGCAC GGATTTATTC TCACATGTTT TGAGACGCCA GAAATCTGAC
```


FIG.10A

```
1151  ACCAGTTTCA ATGTTTAGAC TTGATGCACA CCTGTAATTC TGGTACTTAG
1201  GAGGCAGATG CAGGGGGACT ATGATTTAAA GCCCATTTTT AAGCTGCTGG
1251  GTGAGAACCT GTCTTGATTT TTTTTTCACA TTGGGCTAAA AGTCAAGGAT
1301  CATCAGGGTT GGTGCATTCT GGAAGAAACC TTTGCCTTGC AGCTTCCCAG
1351  AGGGCCGCCA GCATTCCTTG GCTTGTGTTT GGTCCTGGAA TCACTGTGAC
1401  CTTATGCTCC ATCCTCACAT TCCCTCTGCA TTTATCCTCT AAGCACCGGT
                                                  JP.S6
1451  GTGCTTGTAT CCAACCTTTA GGAGCCCCAT AGATCCCCCA TTTCTCCTCG
1501  ACTTAATCAC ACCTGTATAA GTACTTTTCA CTCTGCAAAG CAATATTTGT
1551  GGGTCCAAGG GATTAGGATG TGGGTATATT TGTGGGGTGT CATTATTCAA
1601  TGCTTCATAT TTACACTGTT TCTCTGTTTC ACTTTATTGG GGTACTTGAA
1651  CTTCTAAGAA GAACTGAGGG GTATTGTTGT AGGAACTAAA TTCCCCCATG
1701  GACCTCTGTG CTTTCCACCT ATCACACAAG ACAGAGGGTA TTTGTATTTT
1751  TAGATCCCCA GAAGAAATTC CCACTCTCAA CCCTCCATCC CTGACTTGCT
1801  CACATCTAGA TGAAGCAGGG AACAGCCTGA GNCCTGGAAC TCACTGGAGC
1851  CAGATGACTC TATGGAGTTA GGTTTTAGTA TTCAAGACAC GATGCAAGAC
1901  TCACCTGCCT TCCCCTCACA GACATGTGGC TGCCTGTCAA AGGTGGGGCC
1951  ATGGGGCTGC TGAGACTAAG TCACGTGGAC AGCGCCCATG ACAAGCAGTG
          JP.S7
2001  ACATGGAGAC CAAGGCTGCA GTGTGCATGC TCCACAGGTG CACCTGAAGC
2051  CTCAGAGACG GGAAGAGGAG AGGGAGCAGA AAGATGGGGT ACAGATACCC
2101  CTCTGTTAGG AAGGGCTTCA AAACCGTCTT CTAAGTTTTT GATCCTTTTA
2151  AATGTATCCA CCTGTCACTT GACCCTCTCC TGCTCTGTCT GATCAGCTTC
2201  TCAAAACCCT TCATCCCCTT AACTCCACCC TACTGAAAAA AGATGAAACC
2251  ACTTGTCAAT ATAAACCTCA ACAGCTAAGC ATGGAATACT GTTAACCCCT
2301  CAAGACATAA AGCTGACTGA AGGGATAAGT TTGAAAAAAA TGGGCTTCAG
2351  TTTGCACTAG CTAAGTATGT AACCTTGAAG ATATTACTCA GTTTCTCTGA
2401  ACTTCAGTCT GCTCTCCTAT TTATTGACAA CATGTAAGAG CACATACCGG
2451  GCATTTCTTG TCACCAAATG AAGTTTCCAG TACCAGGAAT GGGTTATATC
```

FIG.10B

2501 TAATCGAGTT GTTGGCCAAA GGAGTTCCAT GGAAACTCCC AAACAATCCA

2551 GGCTATTGGC AAGACTTTTG ATGTCTCTCC ACAAACTGAC AGCAACTGTT

2601 GAAAGACAAT ACCTACACAG CTCACTGAAC ACAGAGAAGC TGAGTTGGTG

2651 CCTACATAAA TCCTCTAGCT CTATGAAGGT CCATAATGGT ATTCATGGCC

2701 CTAGAAGATA CTCTTCCCTC CACCAAAGGA GAAATGTAAA CACTAAGCCA

2751 GCCATAAACC CTTTGGTCTG TTAGAGTGGC CTGCCTGCAA GTTCTGCTGG

2801 TGTAATAATG GCACAGAGCT TGTAGGAGTA ACCAAACAAT ATCTGATAGG

2851 TTAAGGCCCA CTCCATGAGA TCAAACCCAG ACCTAACAAC ACTTGGGTGG

2901 ATGAGAACCC GAGACCAGAT AGGCCAGGGA CCTATGGGAA AACTAAACAT

2951 GACTGTTCTG CTAAAAGAAC CTACCAATAA AATAGCTCCT AGTGACATTC

3001 TGCCATATTT ATAGATCAGT TCCTTGTTCA TCCATCATCA GAAAACTTCC

JP.AS14

3051 TCTTCAGTAG ATAGAAACAA ATATAGAGCC CACAGCCAGA TAATATCCAG

3101 AGAGTGAGAT ACCCTGGAAC ACTCAGCTCT AAAAGGGATG TCTCCATCAA

3151 CCCCCCCCCC CCCCACCTTT CAGGACTCAT GAAACCCTCC AGAAGACGAG

3201 TCAGAAAGAG TGTAAGATCC AGAAGGGATG GAGGACATCC AAAACTTAAG

3251 GCCTTCAAGA CACAACTGTA AGGGAACACA TATGAACTTA GAGAGATGGT

3301 GCAGCATGCA CAGAGCCTGC ATGGGCTTGT ACCAGATGGG GTTCTAGAGC

3351 TGAAAGGAGA AATGGATAGC CACTCTGATT CCTAACCCAG AAGTGACCCC

3401 TAACTGATAG TGACTTGCAA ATAAAAAATT AGTCTTTTTT CAAAGGGAGT

3451 CTCACTGGGA AAATAAACCA CTCTAAATAG TAGACCCCAT GCCCAGCAGT

3501 AGATGGCCAA CAGAAAATGA ACTCAATGTC ATCTTTGACC TTCCTTTGTC

3551 GGAAAGCTTT TTGTTTGCTT TTTCTTACCC TACAGGTCCT TTGCATATTT

JP.AS13

3601 ATTATGGTTT CTTGTTTCAG GTTTTTAATG GAACTCCTGA GTGTGTGAAT

3651 GTGTGTGTCT CTGCATACAT GTGTGTTTCT TAAGCCCGTT CTTTTTCTTT

3701 TCTTCTCTTT ATTGTTTAAA AAAACAATTG TTCTTTATTT TATTATTATT

3751 CCTTATTTTA GACAGAAACA TTGTGGATCC AGATGGGAGA AGAGGTTGGA

3801 GGAATTGGGA GGAGTAAAGG GACAGAAACC ATAATCAGGG GGAACCATAA

FIG.10C

3851 TCAGGGAGAA CCATAATCAG GGGGAGCCAT AATCAGGGGG AGCCATAATC

3901 CAAGGGAACC ATAATCAGAA TATACTGTAT GAAAAAAATT CTATTTTCAA

3951 TAAAAAAAGA ATAAAAAAAA AACAGTCTGA CTGAAGAATA GCACTTGGTA

4001 AGTAACTCTT GTTATAACAA TCCATATCAA ATGCCCTGCC TGTGTTAGCA

4051 AGTTAAGAGA AAAGATTATT CCAAGAGATC CAAGTCTCCT TCAAAACCAA

ZT.S1

4101 GTGTGTACAG AACATTGTCT GAGGAGTAAG ATTGCATTTG GCAACATGCA

4151 TGTCTTTAAT GGTGTGGAGA ATTTCAGTGG AGTTGGCACG TCAGAAAGCA

JP.AS12

4201 CACTGGTGAA AAATGGAGAG AATAGATATA TCCTTTGAGA AATTTGGTCT

4251 CAAAAAGTAG GGTATCAAAT TACTTGGTGT CTGTGAGATC AATTGGTTGT

4301 CTCTGTAGGT TAGCTTACAT AGGAGACAGG AATAAGTGAA GGAGAGAAGG

4351 GAGGACATTG GAGCACCCAA GGAGAGAGGG ACCTTCCTCC TAAAAGTGAA

4401 TGAGGTGGCC TTCATTCCAA GGAGAAGAGA TTCAGGTCGC CCGGGAAGAT

4451 GAGGGACCAA CATCCACAAG GAATGGCAGG AAGTCATCCT GTGTGCATAA

4501 ATGGAGAGAG GGGGTCAAAG ATGGAGCAAA GAAGGATGAG CAAGAAAATG

4551 GTGGATGTGG ATACTCTGAG GATGGCCTGG CTGTGGTGAG CAAAATGTGG

4601 GCAAAGTGGC ACTCCATGAA CAAGACAGCT TGCTCTGTTT GCAGATCCTT

4651 AAATAAAGGC ACATGGCATG CCATGGAGGC TAGGGGAGTG GAGGGGAAAG

4701 GTATATAGAT AGATGCAGAA GTACCAGAGG AGCCAGGAAG GACAGGAGTA

4751 GGAGGGACAG GTTTGCACAA GGCTTTGTCC TCTCCCCACC AGCTCTCTCT

JP.AS11

4801 CCCTTCTGTA TATGCACATA CACAGTGAGC TAGTGTGCAT ATGTGTGCAC

4851 ATATGCATGT GATGAACAGA GGCCAGTCTT GGGTGTCAGT CTTCAGGCCC

4901 TATCTACCTT GTTTTTGAGA CAATCTCACT TGAGTGAGTT GAGTGACTCT

4951 CCTAGTATTC TACAGAGGTT TCCTCAGGTG GGGAGGAATG GGTGGGAGAA

5001 GCAAATTTAA GACTGGTTGA TTTCTTGAAT TTCAGTGGGC TTGGGAAATA

5051 GCAGCTATAT ATTCAGTTTC CTCGTTCCTG GCTGGCTTCC TGGGGTGATC

5101 AGAGCAGAGT ATAGTAGCCC TGTGTGGCAG TCACACCAAG CAGACAGAAG

5151 ATAGGGCATG GCTCTGGTGT GGCTGGTAGA CATAGGAAAG GATCCTTGTA

FIG.10D

```
5201  GCAAGATGTT TGCCATCTCC AGAGACTTAG ACAGCCCAGG AAAGTTTGTC
5251  CTCCCAGGAC CAGCCAGCAC TGAGACTGGA ATGCATCAAA TCCAGAGACC
                                  JP.AS10
5301  AGAAAGCACG GTGCTAGCAC TTAGGAAGAG ACACTAGCCC AAAGTCTCCT
5351  TGCTCCTGCC TAAAGCTTTG CCAATTCTGC AAACCTTGAA AAATTAGCAT
5401  CTTTAAATTC AGAAGGGATA CAAGAAGAGA ACTTACATGG GACCTTGTAA
5451  AAAAGCATAG GGCATCAGTA ACTAAAGTTA CAAAGATAAC AATCAGTGGT
                ZT.S2
5501  GAGTGAACAA AGGACATGGC CATGTTTTTT TTGTTATGAA ACACACGCAC
5551  AGGCACAGGC ACTCACGTGT GCGCACGCGC GCACACACAC ACACGCGCAC
5601  ACACACACAC ACGCATGCAC ACATGCACCA CACACAAACT GCAAAAGTGA
5651  ATAAAAAGAT ATTTCTCACT TTGGCAAAGT GGATGGAAAG TTGATCAAAA
5701  TGAAAGTTAT ACTCAGAACT ATTTTGTACT AGAGGGAGGT TATAAATTAT
                                        JP.AS9
5751  TGTTATTGTT ATATTCTATT TTACTGTTTG TGGCAGCCTA AGTTGGTCTT
5801  GAACTCACTA TGAAGCTAGC AATGACCTTG AGCTTCTGAT CCTTATATCT
5851  ACACTCTCAA GTGCCCAGAT TATAAGTGTG CACCACTATA CTCAGTTTAT
5901  GCTGTGCTAA GGACTAAGCC CAATTATACA AACACACACA CATATATACA
5951  CACATACACA CACACACACA CGTATATATA TGTATATATA TATACATACA
6001  TACACACACA CACACACATA TATGTAAAAT TTGGGAAGAT ATATCAATCT
6051  TCTTTAAAGT ACATGCTACT TTGGTCCAAA ACTTTCACTT TTAGGAAGTT
6101  AAGAAGGAAG AGACAGAATA AGAGATGTCC CAAGAAAGTC AGTGTGGTTG
6151  TCTTAGTTAT GCTTCCTGCT CAGTCAATGT TTCAGATTTT TCTCAGCACA
                JP.AS8
6201  ATGACATCTA TTCTATCAAG TTTTTGATAA CTCTTTACAT GGGACTGGGT
6251  GTGGCTTGTG GCTCTAGCTA TTTCTATTTG TGACTGCCTA TCAGCAAAGC
6301  ATCCACTTCA GACTTTGACT CAAACATCAC CAAGTATTCC CACTTGCATT
6351  GTCTCTGTTA ACCAGCATCA CTGTTCACAG GGCAGGGCAT CACATCTCAC
6401  AAAGGGAAAG GGAAAGGGAA GAGTTAAATT CCCTGGGATA CTAGTCACGG
6451  TGGACTCAGG CAAACAGCCT CTTCAATTGT AAGATGATTC CCTAGTCCAA
                                              JP.AS6
6501  GGACCCTCTA CTGTTTGGAC TCCAGTCTTG TCTGACAGAG GTCCAGTTCA
```

FIG.10E

6551 GGAGTGTCCA GATGGTCTGA TAACCTGATG CCATTCTCAG AGACTCTTTC

6601 CTGTCTGGAA TCTAGTGAGG AGGACTTATC TGGTGAAGCT GTCCTTTAGA

6651 ACAGGAGTGT GTTCCAGTCT TCAAAGCAAA CATTCCTTTT ATCCTAACAC

6701 AGTCTGACTT CAGATATACT GTCTTTTTCC TGGCTCCTTG GGCTTAGGTC

6751 TACCTTGTCC TTGCCCAGGT CCAAGAAAAG GCCCAGAACC TTGGCACTGT

6801 TTTGCCAGTT AATGTCTAAC TGAGGAATGT CTTGCTGCCA AAAGGTGAAA

6851 ACAGAGACCT TGTATTTCCA GGCACAGGTG TGACCCCAAT GTCAATCATT

6901 TTGTGTCTAA CTCCCAGGGG AAAAACTAAC AACAACAGAC TCATGGCTTG

ZT.S3

6951 GAAAAGGTGA ATTCTATGCC AAAAGGGAAG GAAAGTTCTA CCCCCACAGA

ZT.S4

7001 AACAATCTCA GAGGGCAGAA GCAGAGAATA ATCTGAGGGA GAGGGCCAGC

7051 CAAGGGCAGG CAAGTATATA TTGATCACAG GCACTTACTT GTGA**ATGGAC**

EXON 1

7101 **CAGTCCTGTC CTGG**GTTCAG GTAAGGCTGT ATGAAACTGT CACCCCCATA

JP.AS15

7151 TCCACTTCTC CTCTATCTAA TCCCATTATA TTTCAGGGAG GTTGTGGTAG

JP.AS4

7201 AAGCTTAGCT TCTGGACACT GGGGTCCCAT GCTAACCTTC ATGGCATCCT

7251 GGTATGCTGC TGTAAAACCT AGGGTAATGC TTGCATCCAT CTGGAATTAT

7301 TTCACCTGTT GCAACCACAA TCATTTTGAA AATACTAGTA TGTATTATAG

7351 TTATGTATGT ATATAGAGTT AATCATCTCT AAAGCTCCTT ATCTTTTGCC

7401 ATTTCTTTAC ATGAGTTGTA TGAAGATGTA GACGATATTC ATTATTCTCT

7451 TTGGTATCTA GCACCTTGTT TGGCACATAA TACTACTCAA TAAGGGTTTG

7501 TTGAATGAAT AAGTAGGTGA GAGCAAATTG TAAGTTCAGG TAATCACGAA

7551 CTTCCTGTAA AACTCCAAGG CTGCCTCCAG TAAGGTATAA GTCCTGAGTG

7601 AGCCTTTCCC CATCTTGCAA CTTTTTGCTC CAAATGAAAG ACTCAGTTCT

7651 TCAAAATGTG CAGCACATGG AGGTTTGCGA CATAGGGGTG TATTCACAGA

7701 GGCTTCGGAA GCCCACCAAA CCTACAGTTA GATCACTGTA CAGTCTTCCT

JP.AS2

7751 TTTACATACA AGCTGTGCCT CCTGGTNTAC ATCCATGCTG TTTTCTGATC

7801 CATATAGAGG GTACACAACA AAAGCATTTC TTCTGTCTAT AGGGAAGCAA

7851 ATTAGATCAT GCATGTGCCT CACCCACCTC TGTTCTCATG AT**TTCAGGCA**

FIG.10F

EXON 2

7901 **TCAGAAACAC AAGGGAAATC CAAAGTACCT AACCCATCCT TGCCTTTGGG**

7951 **CAGGTGTTTC CAGGACAGAG GGCAGAGTGT AAAGGATGGG GATCCCTTTG**

8001 **ACCTGGATGC TGCTGGTAAT GATGGTAACC TCCTGGTTCA CTCTGGCTGA**

8051 **AGCCAGTAAC TCAACAGAAG CGA**GTAAGTG TGTGTGTGTG TGTGTGTGTG

8101 TGTGTGTGTG TGTGTAGAGA AATGTTCCCT TTGCAGAAGC AATCTTAATC

JP.S1

8151 CCTCTTTTAG CACACTTGAT GTGATCTTTA TTTTAAGCCC ATTTCTCAGA

8201 TTGTAATGAG CACAGGACTC ACTTCGAAGT TTTGTTAAGA TGCAAATTCT

8251 ACTTTAGTAG GTCTAGCAAG GGG/CCCGAGA CTCTGAATTA ATAGCAGCGT

APA/KPN JUNCTION

8301 GTGGGTGATG TTTCTGGTGG GACAAGGGGC TAAAACACCT CTGAACCATT

8351 TCTGCACTTC ACGGTAAAGT CACAAGCATG CCCAGATACA TAAGAGATTT

8401 GACCCACCTC TCCTGTAAGT GTGAAGTCAT CCCATGGGGG TAGCTTTGCC

8451 TTCCACCCTG GAGTACTCTG GAATTACACT AAGTATAATT GTGAGGTCAT

8501 GGTTAAAAGC ACATGTTCTG TGGTCAGGCC ATGTGCGTGT ACCCTGTTTG

8551 ACAACTGGCT TGCTCGTTCT GAATGTCAAT ATTCTTTTCT GTAAATGAAG

8601 AAAATGAAAA TGGGTTCCAG CGGCAGGGGG TGTGCCCTGG GGAGGATTCG

8651 CTAAACTCTA GACTGAAAAG TCAATGAATA GAGGACTCCA CTCAGGGGAG

8701 CTCGGATGGG TGTGTTTTGA AGGTGCCAAC AACTTAACAA GTCCAGAAAA

8751 GCAAGAAAGT ATGGGCAGGG GCACCTGCCA GCTGCAGGGA TTCTGAAGCT

JP.AS5

8801 GGGCTCTTCT GTCCGCAG**GA CGGTGTTCTG AATGCCACAA CAACGCCACC**

EXON 3

8851 **TGCACGGTGG ATGGTGTGGT CACAACGTGC TCCTGCCAGA CCGGCTTCAC**

8901 **TGGTGATGGG CTGGTGTGTG AGGACATGGA TGAGTGTGCT ACCCCATGGA**

8951 **CTCACAACTG CTCCAACAGC AGCTGTGTGA ACACCCCGGG CTCGTTTAAG**

9001 **TGCTCCTGTC AGGATGGTTT TCGTCTGACG CCTGAGCTGA GCTGCACTGA**

9051 **TGTGGATGAG TGCTCAGAGC AGGGGCTCAG TAACTGTCAT GCCCTGGCCA**

9101 **CCTGTGTCAA CACAGAAGGC GACTACTTGT GCGTGTGTCC CGAGGGCTTT**

9151 **ACAGGGGATG GTTGGTACTG TGAGTGCTCC CCAGGCTCCT GTGAGCCAGG**

FIG.10G

9201 ACTGGACTGC TTGCCCCAGG GCCCGGATGG AAAGCTGGTG TGTCAAGACC

9251 CCTGCAATAC ATATGAGACC CTGACTGAGT ACTGGCGCAG CACAGAGTAT

9301 GGTGTGGGCT ACTCCTGTGA CGCGGGTCTG CACGGCTGGT ACCGG

POLYCLONING SITE OF pBS

FIG.10H

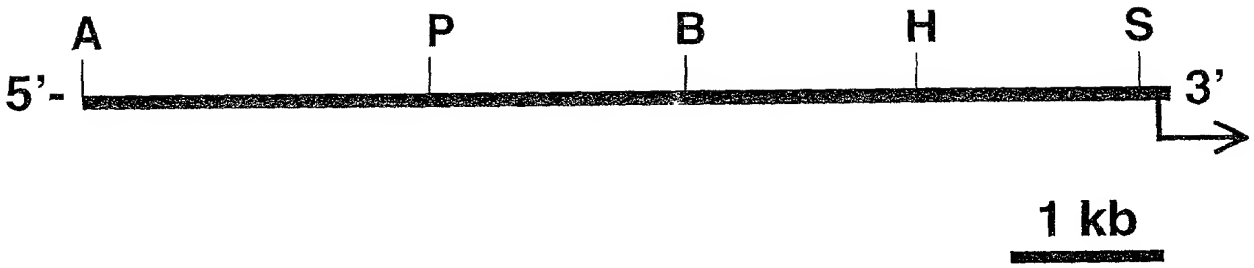


FIG.11

```
  1  TACTGGCGCA GCACAGAGTA CGGCTCCGGC TACGTCTGTG ATGTCAGTCT
                                      <-AS14
 51  GGGCGGCTGG TACCGCTTCG TGGGCCAGGG CGGCGTGCGC CTGCCCGAGA
101  CCTGCGTGCC CGTCCTGCAC TGCAACACGG CCGCGCCTAT GTGGCTCAAC
                                      <-AS15
151  GGCACGCACC CATCGAGCGA CGAGGGCATC GTGAACCGCG TGGCCTGTGC
201  GCACTGGAGC GGCGACTGCT GCCTGTGGGA CGCGCCTGTC CAAGTGAAGG
251  CCTGTGCCGG CGGCTACTAC GTGTACAACC TGACAGAGCC CCCTGAG
     <-AS17
```

FIG.12

```
  1  ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAT CTTAAAAAAA
                                AS1
 51  AAAAAAAACA AAAAGAACAT CACTAAGCCC CCCTGCCCTG GCACTTTATT
                                    AS2
101  GGAAGGTCAA GAACACACTC AACCACACAA GAGATGTGAA CATACCTGTG
                      AS3
151  TGGTACCCAA AGACATCCCC TTTCACACAT ACATGACCCT TCCATTGGGT
                AS4                          AS5
201  TGCACATTGC TGTTAGCTTT TTGTTGGAGA AGGGAGCTAG ACACCTCTAC
251  ACAACCCCCA ACTGGAGTTC TCTGGAACAG AGTAAATACC ATCGTGTCAT
301  CATGGAGCGC ACACACACTG TGGTCCTGCA ACCTCGATTT GTGTCCTGGC
351  TCTGCTGCTT ACCAATGAAG CAAGTAGCTT AAACCTTCTG AATCTCAAGT
401  TTCCTCACCC TCAAACTATA GCTAAATACA AAAGTCATTT CCCAGGGCCA
451  CTGGAGAGGA TTCTATCAGA TAATGGATAG AAGATGCCTA TCCCAGTGTT
501  TGACATATCC TAAGTGCTTA ATACACGAGA GCTCACCATC TTTACTGGTA
551  TTATTGCACA GAGAAACACA CAAAGTGTCA GTGCCCCTGC TAGGTAGAGA
601  GGGANGCANG GNAAGGAGAT CTGAGCAAAA GGCATAGAAT ATATCAAGCT
651  GGG
```

FIG.13A

```
  1  CGGGGGAAGG TTTATTTTGT TTCTTTTCAA AGGGGGTCTT GNTCTGTCTC
 51  AAAGACCNTA AGGACCATGA AAAAATCTCT TTGTNAAAAG TGCCAAGCGG
101  TCCCCACTCT GAATCTGGGC TTTTCTGCCT GCAGAAAGCT GCTCTGAATG
151  TCACGCCAAT GCCACTTGTA CGGTGGACGG GGCTTGCCAC GACCTGCGCC
201  TGCCAGGAGG GCTTCACTGC GACGGCCTCG AATGTGCGGA TCTGGATGAA
251  TGCGCCATTC TGGGGGCGCA CAACTGCTCC GCCACCAACA GCTGCGTGAA
301  CGCGCTGGGC TCCTACACAT GCGTCTGCCC TGAAGGTTTC CTCCTGAGCT
351  CGGAGCTCGG CTGCGAGGAT GTGGACGAGT GTGCAGAGCC AGGGCTCAGC
401  CGCTGCCACG CCCTGGCCAC CTGCATCAAT GGCGAGGGCA ACTACTCATG
451  CGTGTGTCCC GCGGGCTACG TGGGGGACGG GAGGCACTGT GAGTGTTCCC
501  CGGGCTCCTG CGGGCCTGGG CTAGACTGCG TGCGGGAGGG TGACGCGCTA
551  GTGTGCGCTG ACCCGTGCCA GGCGCACCAC ATCCTGGACG AATACTGGCG
601  CAGCACAGAG TACGGCTCCG GCTACGTCTG TGATGTCAGT CTGGGCGGCT
651  GGTAC
```

FIG.13B

```
  1  ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAG ACACCCAGAC
 51  TTAGGTTTTG ACAGAGCCTC ATGTTCACCA ACCAGAAATG ACATTCACCA
101  CCTAGGATTG AGAAAAAGAA TATTAGGAAC TTTTATTTTC TTCTGAAGTT
151  ATAGCAAAGA AAGGGGAAAA AAAAAAACAT TCTTATGGGG GATAAACGGG
201  CAAAGGATAC AAACAGTTCA GAAAAGAATA AATAGTAAGC AAATGAAAAG
251  ATAACTTCCT TTTTCATCAA AGAACCGCAA AAGTAAATAA TGATAAGATG
301  TTTCTCACTT TTCCACAAAG ATGAAAGTTA ATGCCCAGGG TGGCTGAGTA
351  CTGTGCTGGG ATTGTGAACT AACTGTTATA GATCTCTCTG GGGTGCTGTT
401  TGGGAAGAAA CATCGCTGAA AACTGAGCTA CCTCTTTTCC TATGAAATTC
451  CCCTGAGGAG GTGAGTGAGC CGCTGCTGAT CGTCACCCGA GCACTAGGCC
501  AGACAGAAGG AGAAAGCCCT CAAAGAGGCA ATGCTGTGGA TCACTGTCAT
551  ATTTCCTGCT CAGCCTGAGT TCACATGTGC CTGATTTTTC TCAATATGGC
601  ATTGCCATTA ACGTGGAATT AGGTCAGGAG ACCTAAGGCT GAACCAAGCC
651  CTGTCATTCT CTGCCCCATG ACTGCGCATC ACCAAAACAG CATCGGCAGT
701  GACTTCCACA GATGGTACCA TTGCTATATG CCTTAACTTG CATCATCTCC
751  TTTAATGGCC ATAACAATTC TAGGACACGG GTATTCTTGT TTTACAGATG
801  ATGAAAATTA CCTCTGGAAG GAAAATTACT GGCACACAAA AAACGCTGAC
851  CAGGATTCAG ATAGACTGAC TCCAAAGTCA GTCTGTTCAT CTACAAAATT
901  ATCTACTTCT CAAGGACCTT CCTTCATGGG AATTCAAATT TCTTGATTCA
951  CAGAGCATCT GGTCCAATGA TGTCTGAATT ATCTGCTGTC TCTGACCTTC
```

FIG.14A

```
1001  AGCCATTCTC AGCTCCTTTC CTGATCACAT TGGGACCCCA GGGGAGCTGG
1051  CTGAATCTGT GAGGATGGCA TTTGCTTTGG AATTAAGTGG CCACAAGTAC
1101  ACATCCTGGT GGGGACGATG AGCACCCCTT TTCTCCTGGA GCAGCCTGGC
1151  TTCAGATTCT GGCCTCTGCT TGGCTCCACT TTGTGCTTTT CAATGACCAA
1201  GAAAATCCCA GGCCCTTGGA ATTGTTTACT CAGTTAATTT CTAACTAAAG
1251  AACCTCTTGT TGCCAAAAGG TATAAAACAG AGCCCTTGTA GCTGTGGGCA
1301  CAGCTGTGAC CCCCATGTCA ATCATTTGGG GTCTCTACCT ATTAGGGAAA
1351  AGAACAACAA CCACCTCACA GCCTAGAAAA GGAAAACACT GTGTCAAAAG
1401  GGAAAAATAT TCCACCCCCA TTAAAATAAT TAAGAAACAG AACCAGAGGA
1451  TCATTGGAGG AGAGATTGCC AGTGGGGGAC AGATGTATAT ATATAGATAT
1501  GAAAGTCACC TACTTGTAAA AGGATTAATT CTACCTTTCT GGTTTCAGGT
1551  AAGGCTATCT GCAGCTCTCA CTTCTCCTAG CCACTTCTCC CATCTAGTCT
1601  TTGCTGGCTC CCATTCTGTT TGAAGGATGG
```

FIG.14B

```
$ type guromodulinpromoter18full.pair;1
 BESTFIT of: Guromodulinpromoter18full  check: 3852  from: 1  to: 1630


 to: mouseThppromoterfull.  check: 5595  from: 1  to: 9343


 Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
 CompCheck: 2335

         Gap Weight:     50      Average Match: 10.000
      Length Weight:      3   Average Mismatch: -9.000

            Quality:   1617             Length:    534
              Ratio:  3.177              Gaps:     15
 Percent Similarity: 74.385   Percent Identity: 74.385

        Match display thresholds for the alignment(s):
                    | = IDENTITY
                    : =   5
                    . =   1

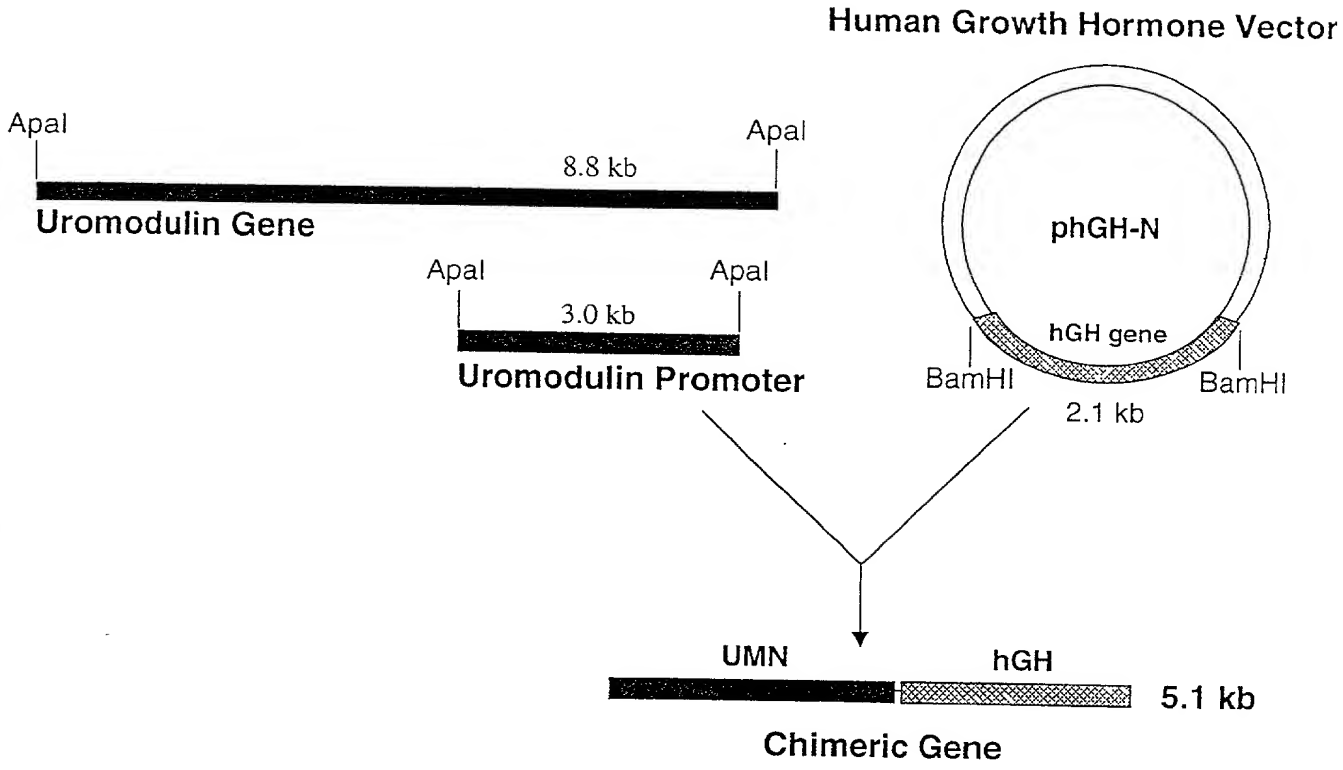
 Guromodulinpromoter18full x Thppromoterfull. March 24, 2000 16:31  ..

                    .          .          .          .          .
    1121 AGCACCCCTTTTCTCCTGGAGCAGCCTGGCTTCAGA.............T 1157
         | ||  |||||| ||||    ||| ||| |||||||             |
    6677 AACATTCCTTTTATCCTAACACAGTCTGACTTCAGATATACTGTCTTTTT 6726
                    .          .          .          .          .
    1158 TCTGGCCTCT...GCTTGGCTCCACTTTGTGCTTTTCAATGACCAAGAAA 1204
          |||||  ||   |||| | || || |||| |||  | | | ||||||||
    6727 CCTGGCTCCTTGGGCTTAGGTCTACCTTGTCCTTGCCCAGGTCCAAGAAA 6776
                    .          .          .          .          .
    1205 A.TCCCAGGCCCTTGGAATTGTTTACTCAGTTAATTTCTAACTAAAGAAC 1253
         |  |||||  |||||| | |||||   |||||||| ||||||| | |||
    6777 AGGCCCAGAACCTTGGCACTGTTTTGCCAGTTAATGTCTAACTGAGGAAT 6826
                    .          .          .          .          .
    1254 CTCTTGTTGCCAAAAGGTATAAAACAGAGCCCTTGTAGCTGTGGGCACAG 1303
          ||||| |||||||||||  |||||||| |||||||  |   |||||||
    6827 GTCTTGCTGCCAAAAGGT.GAAAACAGAGACCTTGTATTTCCAGGCACAG 6875
                    .          .          .          .          .
    1304 CTGTGACCCCCATGTCAATCATTTGGGGTCTCTACCTATTAGGG...AAA 1350
          ||||||||| ||||||||||||    || |||| ||   ||||   |||
    6876 GTGTGACCCCAATGTCAATCATTT..TGTGTCTAACTCCCAGGGGAAAAA 6923
                    .          .          .          .          .
    1351 AGAACAACAACCACCTCACAGCCTAGAAAAGGAAAACACTGTGTCAAAAG 1400
           |||||||||   ||||  || | |||||||  ||  || || ||||||
    6924 CTAACAACAACAGACTCATGGCTTGGAAAAGGTGAATTCTATGCCAAAAG 6973
                    .          .          .          .          .
    1401 GGAA.AAATATTCCACCCCCATTAAAATAAT.TAAGA.AACAGAACCAGA 1447
         ||||  ||  ||| |||||||   ||| ||| | |||   ||||| ||||
    6974 GGAAGGAAAGTTCTACCCCCACAGAAACAATCTCAGAGGGCAGAAGCAGA 7023
                    .          .          .          .          .
    1448 GGATCATTGGAGGAGAGATTGCCAGTGGGGGACAGATGTATATATATAGA 1497
         | || ||  |||| ||||  |||||    || |||  | |  ||||||
    7024 GAATAATCTGAGG.GAGAGGGCCAGCCAAGGGCAG..GCAAGTATATATT 7070
                    .          .          .          .          .
    1498 TATGAAAGTCACCTACTTGTAAAAGGATTAATTCTACCTTTCTGGTTTCA 1547
          || | || ||| ||||||| || |||  | | ||    | |||| ||||
    7071 GATCACAGGCACTTACTTGTGAATGGACCAGTCCT...GTCCTGGGTTCA 7117
                    .          .          .          .          .
```

FIG.15A

```
1548 GGTAAGGCTATCTGCAGCTCTCACTTCTCCTAGCCACTTCTC..CCATCT 1595
     ||||||||| | || | || ||||  | | || |||||||||  | ||||
7118 GGTAAGGCTGTATGAAACTGTCAC.CCCCATATCCACTTCTCCTCTATCT 7166
                .          .          .
1596 AGTCTTTGCTGGCTCCCATTCTGTTTGAAGGATG 1629
     |            ||||||| | ||| | ||| |
7167 A...........ATCCCATTATATTTCAGGGAGG 7189
```

FIG.15B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ApaI
8.8 kb
Uromodulin Gene
ApaI
ApaI
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hGH gene
BamHI
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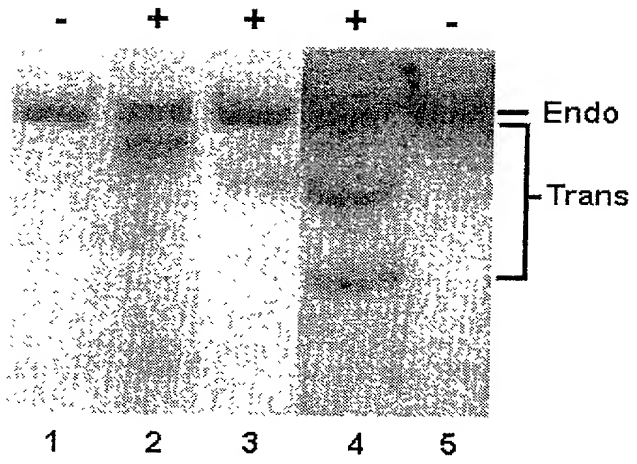
UMN
hGH
5.1 kb
Chimeric Gene

FIG.16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+
+
+
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Endo
Trans
1
2
3
4
5

FIG.17

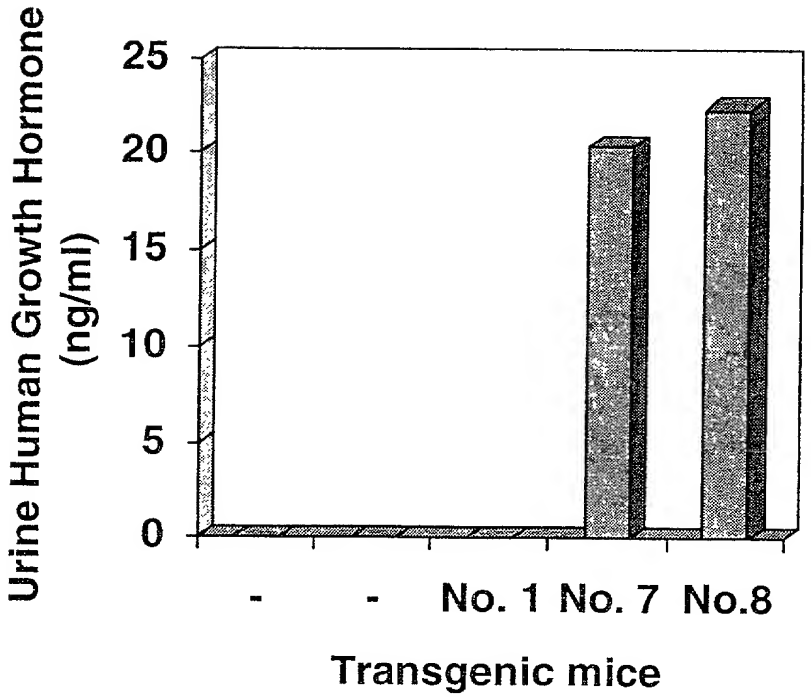
Urine Human Growth Hormone (ng/ml)
25
20
15
10
5
0
-
-
No. 1
No. 7
No.8
Transgenic mice

FIG.18

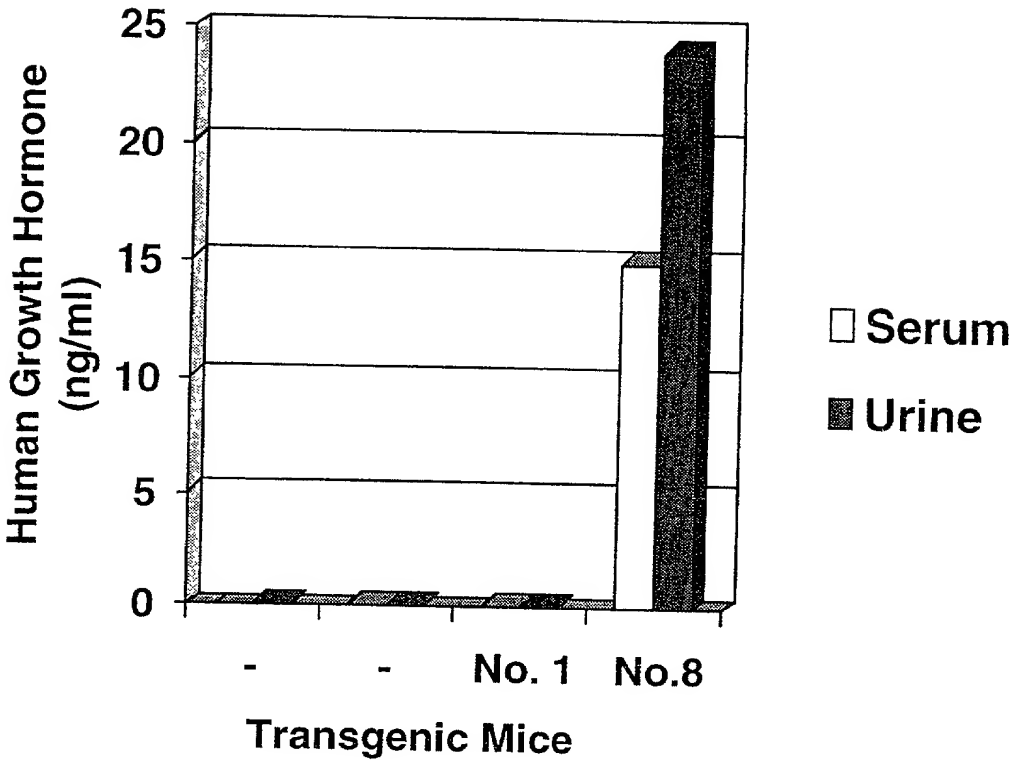
Human Growth Hormone (ng/ml)
25
20
15
10
5
0
- - No. 1 No.8
Transgenic Mice
Serum
Urine

FIG.19

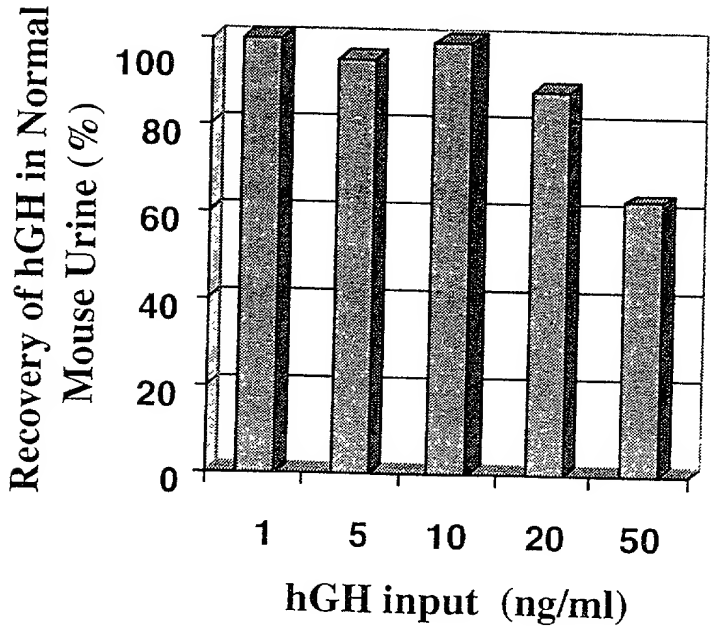
Recovery of hGH in Normal Mouse Urine (%)
100
80
60
40
20
0
1 5 10 20 50
hGH input (ng/ml)

FIG.20

SEQUENCE LISTING

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SUN, Tung-Tien

<120> TRANSGENIC ANIMALS AS URINARY BIOREACTORS FOR THE PRODUCTION OF POLYPEPTIDE IN THE URINE, RECOMBINANT DNA CONSTRUCT FOR KIDNEY-SPECIFIC EXPRESSION, AND METHOD OF USING SAME

<130> WU43C

<140> NOT YET ASSIGNED
<141> 2000-06-26

<150> 60/108,195
<151> 1998-11-13

<150> 60/142,925
<151> 1999-07-09

<150> 09/438,785
<151> 1999-11-12

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<170> PatentIn Ver. 2.1

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Val Thr Thr Cys Ser Cys Gln Ala Gly Phe Thr Gly Asp Gly Leu Val
    50                  55                  60

Cys Glu Asp Ile Asp Glu Cys Ala Thr Pro Trp Thr His Asn Cys Ser
65                  70                  75                  80

Asn Ser Ile Cys Met Asn Thr Leu Gly Ser Tyr Glu Cys Ser Cys Gln
                85                  90                  95

Asp Gly Phe Arg Leu Thr Pro Gly Leu Gly Cys Ile Asp Val Asn Glu
            100                 105                 110

Cys Thr Glu Gln Gly Leu Ser Asn Cys His Ser Leu Ala Thr Cys Val
        115                 120                 125

Asn Thr Glu Gly Ser Tyr Ser Cys Val Cys Pro Lys Gly Tyr Arg Gly
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Asp Gly Trp Tyr Cys Glu Cys Ser Pro Gly Phe Cys Glu Pro Gly Leu
145                 150                 155                 160

Asp Cys Leu Pro Gln Gly Pro Ser Gly Lys Leu Val Cys Gln Asp Pro
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Cys Asn Val Tyr Glu Thr Leu Thr Glu Tyr Trp Arg Ser Thr Asp Tyr
            180                 185                 190

Gly Ala Gly Tyr Ser Cys Asp Ser Asp Met His Gly Trp Tyr Arg Phe
        195                 200                 205

Thr Gly Gln Gly Gly Val Arg Met Ala Glu Thr Cys Val Pro Val Leu
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Arg Cys Asn Thr Ala Ala Pro Met Trp Leu Asn Gly Ser His Pro Ser
225                 230                 235                 240

Ser Arg Glu Gly Ile Val Ser Arg Thr Ala Cys Ala His Trp Ser Asp
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His Cys Cys Leu Trp Ser Thr Glu Ile Gln Val Lys Ala Cys Pro Gly
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Lys Gln Asp Phe Asn Val Thr Asp Val Ser Leu Leu Glu His Arg Leu
325 330 335

Glu Cys Glu Ala Asn Glu Ile Lys Ile Ser Leu Ser Lys Cys Gln Leu
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Gln Ser Leu Gly Phe Met Lys Val Phe Met Tyr Leu Asn Asp Arg Gln
355 360 365

Cys Ser Gly Phe Ser Glu Arg Gly Glu Arg Asp Trp Met Ser Ile Val
370 375 380

Thr Pro Ala Arg Asp Gly Pro Cys Gly Thr Val Leu Arg Arg Asn Glu
385 390 395 400

Thr His Ala Thr Tyr Ser Asn Thr Leu Tyr Leu Ala Ser Glu Ile Ile
405 410 415

Ile Arg Asp Ile Asn Ile Arg Ile Asn Phe Glu Cys Ser Tyr Pro Leu
420 425 430

Asp Met Lys Val Ser Leu Lys Thr Ser Leu Gln Pro Met Val Ser Ala
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Leu Asn Ile Ser Leu Gly Gly Thr Gly Lys Phe Thr Val Gln Met Ala
450 455 460

Leu Phe Gln Asn Pro Thr Tyr Thr Gln Pro Tyr Gln Gly Pro Ser Val
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Met Leu Ser Thr Glu Ala Phe Leu Tyr Val Gly Thr Met Leu Asp Gly
485 490 495

Gly Asp Leu Ser Arg Phe Val Leu Leu Met Thr Asn Cys Tyr Ala Thr
500 505 510

Pro Ser Ser Asn Ser Thr Asp Pro Val Lys Tyr Phe Ile Ile Gln Asp
515 520 525

Arg Cys Pro His Thr Glu Asp Thr Thr Ile Gln Val Thr Glu Asn Gly
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Glu Ser Ser Gln Ala Arg Phe Ser Ile Gln Met Phe Arg Phe Ala Gly
545   550   555   560

Asn Ser Asp Leu Val Tyr Leu His Cys Glu Val Tyr Leu Cys Asp Thr
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Met Ser Glu Gln Cys Lys Pro Thr Cys Ser Gly Thr Arg Tyr Arg Ser
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Gly Asn Phe Ile Asp Gln Thr Arg Val Leu Asn Leu Gly Pro Ile Thr
        595   600   605

Arg Gln Gly Val Gln Ala Ser Val Ser Lys Ala Ala Ser Ser Asn Leu
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Leu Met Val His

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<212> PRT
<213> MOUSE UROMODULIN

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Ser Glu Cys His Asn Asn Ala Thr Cys Thr Val Asp Gly Val Val Thr
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Thr Cys Ser Cys Gln Thr Gly Phe Thr Gly Asp Gly Leu Val Cys Glu
     50   55   60

Asp Met Asp Glu Cys Ala Thr Pro Trp Thr His Asn Cys Ser Asn Ser
 65   70   75   80

Ser Cys Val Asn Thr Pro Gly Ser Phe Lys Cys Ser Cys Gln Asp Gly
                 85   90   95

Phe Arg Leu Thr Pro Gly Leu Gly Cys Thr Asp Val Asp Glu Cys Ser
100 105 110

Glu Gln Gly Leu Ser Asn Cys His Ala Leu Ala Thr Cys Val Asn Thr
115 120 125

Glu Gly Asp Tyr Leu Cys Val Cys Pro Lys Gly Phe Thr Gly Asp Gly
130 135 140

Trp Tyr Cys Glu Cys Ser Pro Ser Ser Cys Glu Pro Gly Leu Asp Cys
145 150 155 160

Leu Pro Gln Gly Pro Asp Gly Lys Leu Val Cys Gln Asp Pro Cys Asn
165 170 175

Thr Tyr Glu Thr Leu Thr Glu Tyr Trp Arg Ser Thr Glu Tyr Gly Val
180 185 190

Gly Tyr Ser Cys Asp Ala Gly Gln His Gly Trp Tyr Arg Phe Thr Gly
195 200 205

Gln Gly Gly Val Arg Met Ala Glu Thr Cys Val Pro Val Leu Ala Cys
210 215 220

Asn Thr Ala Ala Pro Met Trp Leu Asn Gly Ser His Pro Ser Ser Ser
225 230 235 240

Glu Gly Ile Val Ser Arg Thr Ala Cys Ala His Trp Ser Asp His Cys
245 250 255

Cys Arg Trp Ser Thr Glu Ile Gln Val Lys Ala Cys Pro Gly Gly Phe
260 265 270

Tyr Ile Tyr Asn Leu Thr Glu Pro Pro Glu Cys Asn Leu Ala Tyr Cys
275 280 285

Thr Asp Pro Ser Ser Val Glu Gly Thr Cys Glu Glu Cys Arg Val Asp
290 295 300

Glu Asp Cys Ile Ser Asp Asn Gly Arg Trp Arg Cys Gln Cys Lys Gln
305 310 315 320

Asp Ser Asn Ile Thr Asp Val Ser Gln Leu Glu Tyr Arg Leu Glu Cys
325 330 335

Gly Ala Asn Asp Ile Lys Met Ser Leu Arg Lys Cys Gln Leu Gln Ser
340 345 350

Leu Gly Phe Met Asn Val Phe Met Tyr Leu Asn Asp Arg Gln Cys Ser
355 360 365

Gly Phe Ser Glu Ser Asp Glu Arg Asp Trp Met Ser Ile Val Thr Pro
370 375 380

Ala Arg Asn Gly Pro Cys Gly Thr Val Leu Arg Arg Asn Glu Thr His
385 390 395 400

Ala Thr Tyr Ser Asn Thr Leu Tyr Leu Ala Asn Ala Ile Ile Ile Arg
405 410 415

Asp Ile Ile Ile Arg Met Asn Phe Glu Cys Ser Tyr Pro Leu Asp Met
420 425 430

Lys Val Ser Leu Lys Thr Ser Leu Gln Pro Met Val Ser Ala Leu Asn
435 440 445

Ile Ser Leu Gly Gly Thr Gly Lys Phe Thr Val Arg Met Ala Leu Phe
450 455 460

Gln Ser Pro Thr Tyr Thr Gln Pro Tyr Gln Gly Pro Ser Val Met Leu
465 470 475 480

Ser Thr Glu Ala Phe Leu Tyr Val Gly Thr Met Leu Asp Gly Gly Asp
485 490 495

Leu Ser Arg Phe Val Leu Leu Met Thr Asn Cys Tyr Ala Thr Pro Ser
500 505 510

Ser Asn Ser Thr Asp Pro Val Lys Tyr Phe Ile Ile Gln Asp Ser Cys
515 520 525

Pro Arg Thr Glu Asp Thr Thr Ile Gln Val Thr Glu Asn Gly Glu Ser
530 535 540

Ser Gln Ala Arg Phe Ser Val Gln Met Phe Arg Phe Ala Gly Asn Tyr
545 550 555 560

Asp Leu Val Tyr Leu His Cys Glu Val Tyr Leu Cys Asp Ser Thr Ser
565 570 575

Glu Gln Cys Lys Pro Thr Cys Ser Gly Thr Arg Phe Arg Cys Gly Asn
580 585 590

Phe Ile Asp Gln Thr Arg Val Leu Asn Leu Gly Pro Ile Thr Arg Gln
595 600 605

```
Gly Val Gln Ala Ser Val Ser Lys Ala Ala Ser Ser Asn Leu Arg Leu
    610                 615                 620

Leu Ser Ile Trp Leu Leu Leu Phe Leu Ser Ala Thr Leu Ile Phe Met
625                 630                 635                 640

Val Gln
```

<210> 40
<211> 640
<212> PRT
<213> HUMAN UROMODULIN

<400> 40

```
Met Gly Gln Pro Ser Leu Thr Trp Met Leu Met Val Val Val Ala Ser
  1               5                  10                  15

Trp Phe Ile Thr Thr Ala Ala Thr Asp Thr Ser Glu Ala Arg Trp Cys
             20                  25                  30

Ser Glu Cys His Ser Asn Ala Thr Cys Thr Glu Asp Glu Ala Val Thr
         35                  40                  45

Thr Cys Thr Cys Gln Glu Gly Phe Thr Gly Asp Gly Leu Thr Cys Val
     50                  55                  60

Asp Leu Asp Glu Cys Ala Ile Pro Gly Ala His Asn Cys Ser Ala Asn
 65                  70                  75                  80

Ser Ser Cys Val Asn Thr Pro Gly Ser Phe Ser Cys Val Cys Pro Glu
                 85                  90                  95

Gly Phe Arg Leu Ser Pro Gly Leu Gly Cys Thr Asp Val Asp Glu Cys
            100                 105                 110

Ala Glu Pro Gly Leu Ser His Cys His Ala Leu Ala Thr Cys Val Asn
        115                 120                 125

Val Val Gly Ser Tyr Leu Cys Val Cys Pro Ala Gly Tyr Arg Gly Asp
    130                 135                 140

Gly Trp His Cys Glu Cys Ser Pro Gly Ser Cys Gly Pro Gly Leu Asp
145                 150                 155                 160

Cys Val Pro Glu Gly Asp Ala Leu Val Cys Ala Asp Pro Cys Gln Ala
```

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                165                 170                 175

His Arg Thr Leu Asp Glu Tyr Trp Arg Ser Thr Glu Tyr Gly Glu Gly
            180                 185                 190

Tyr Ala Cys Asp Thr Asp Leu Arg Gly Trp Tyr Arg Phe Val Gly Gln
        195                 200                 205

Gly Gly Ala Arg Met Ala Glu Thr Cys Val Pro Val Leu Arg Cys Asn
    210                 215                 220

Thr Ala Ala Pro Met Trp Leu Asn Gly Thr His Pro Ser Ser Asp Glu
225                 230                 235                 240

Gly Ile Val Ser Arg Lys Ala Cys Ala His Trp Ser Gly His Cys Cys
                245                 250                 255

Leu Trp Asp Ala Ser Val Gln Val Lys Ala Cys Ala Gly Gly Tyr Tyr
            260                 265                 270

Val Tyr Asn Leu Thr Ala Pro Pro Glu Cys His Leu Ala Tyr Cys Thr
        275                 280                 285

Asp Pro Ser Ser Val Glu Gly Thr Cys Glu Glu Cys Ser Ile Asp Glu
    290                 295                 300

Asp Cys Lys Ser Asn Asn Gly Arg Trp His Cys Gln Cys Lys Gln Asp
305                 310                 315                 320

Phe Asn Ile Thr Asp Ile Ser Leu Leu Glu His Arg Leu Glu Cys Gly
                325                 330                 335

Ala Asn Asp Met Lys Val Ser Leu Gly Lys Cys Gln Leu Lys Ser Leu
            340                 345                 350

Gly Phe Asp Lys Val Phe Met Tyr Leu Ser Asp Ser Arg Cys Ser Gly
        355                 360                 365

Phe Asn Asp Arg Asp Asn Arg Asp Trp Val Ser Val Val Thr Pro Ala
    370                 375                 380

Arg Asp Gly Pro Cys Gly Thr Val Leu Thr Arg Asn Glu Thr His Ala
385                 390                 395                 400

Thr Tyr Ser Asn Thr Leu Tyr Leu Ala Asp Glu Ile Ile Ile Arg Asp
                405                 410                 415

Leu Asn Ile Lys Ile Asn Phe Ala Cys Ser Tyr Pro Leu Asp Met Lys
```

420 425 430

Val Ser Leu Lys Thr Ala Leu Gln Pro Met Val Ser Ala Leu Asn Ile
435 440 445

Arg Val Gly Gly Thr Gly Met Phe Thr Val Arg Met Ala Leu Phe Gln
450 455 460

Thr Pro Ser Tyr Thr Gln Pro Tyr Gln Gly Ser Ser Val Thr Leu Ser
465 470 475 480

Thr Glu Ala Phe Leu Tyr Val Gly Thr Met Leu Asp Gly Gly Asp Leu
485 490 495

Ser Arg Phe Ala Leu Leu Met Thr Asn Cys Tyr Ala Thr Pro Ser Ser
500 505 510

Asn Ala Thr Asp Pro Leu Lys Tyr Phe Ile Ile Gln Asp Arg Cys Pro
515 520 525

His Thr Arg Asp Ser Thr Ile Gln Val Val Glu Asn Gly Glu Ser Ser
530 535 540

Gln Gly Arg Phe Ser Val Gln Met Phe Arg Phe Ala Gly Asn Tyr Asp
545 550 555 560

Leu Val Tyr Leu His Cys Glu Val Tyr Leu Cys Asp Thr Met Asn Glu
565 570 575

Lys Cys Lys Pro Thr Cys Ser Gly Thr Arg Phe Arg Ser Gly Ser Val
580 585 590

Ile Asp Gln Ser Arg Val Leu Asn Leu Gly Pro Ile Thr Arg Lys Gly
595 600 605

Val Gln Ala Thr Val Ser Arg Ala Phe Ser Ser Leu Gly Leu Leu Lys
610 615 620

Val Trp Leu Pro Leu Leu Leu Ser Ala Thr Leu Thr Leu Thr Phe Gln
625 630 635 640

<210> 41
<211> 459
<212> PRT

<213> BOVINE UROMODULIN

<400> 41

```
Met Lys Cys Ser Asn Met Trp Met Ala Ala Val Val Thr Ser Trp Val
  1               5                  10                  15

Ala Ala Thr Asp Thr Ser Ser Ala Lys Ser Cys Ser Cys His Ser Asn
             20                  25                  30

Ala Thr Cys Thr Val Asp Gly Ala Ala Thr Thr Cys Ala Cys Gly Thr
         35                  40                  45

Gly Asp Gly Cys Val Asp Asp Cys Ala Val Gly Ala His Asn Cys Ser
     50                  55                  60

Ala Thr Lys Ser Cys Val Asn Thr Gly Ser Tyr Thr Cys Val Cys Gly
 65                  70                  75                  80

Ser Ser Gly Cys Asp Val Asp Cys Ala Gly Ser Arg Cys His Ala Ala
                 85                  90                  95

Thr Cys Asn Gly Gly Asn Tyr Ser Cys Val Cys Ala Gly Tyr Gly Asp
            100                 105                 110

Gly Arg His Cys Cys Ser Gly Ser Cys Gly Gly Asp Cys Val Arg Gly
        115                 120                 125

Asp Ala Val Cys Val Asp Cys Val His Arg Asp Tyr Trp Arg Ser Thr
    130                 135                 140

Tyr Gly Ser Gly Tyr Cys Asp Val Ser Gly Gly Trp Tyr Arg Val Gly
145                 150                 155                 160

Ala Gly Val Arg Thr Cys Val Val His Cys Asn Thr Ala Ala Met Trp
                165                 170                 175

Asn Gly Thr His Ser Ser Asp Gly Val Asn Arg Val Ala Cys Ala His
            180                 185                 190

Trp Ser Gly Asp Cys Cys Trp Asp Ala Val Lys Ala Cys Ala Gly Gly
        195                 200                 205

Tyr Tyr Val Tyr Asn Thr Ala Cys His Ala Tyr Cys Thr Asp Ser Ser
    210                 215                 220

Val Gly Thr Cys Cys Arg Val Asp Asp Cys Lys Ser Asp Asn Gly Trp
225                 230                 235                 240
```

```
His Cys Cys Lys Asp Asn Val Thr Asp Ser Arg Arg Cys Gly Val Asp
                245                 250                 255

Asp Lys Ser Ser Lys Cys Lys Ser Gly Lys Val Met Tyr His Asp Ser
            260                 265                 270

Cys Ser Gly Thr Arg Gly Asp Arg Asp Trp Met Ser Val Val Thr Ala
        275                 280                 285

Arg Asp Gly Cys Gly Thr Val Met Thr Arg Asn Thr His Ala Thr Tyr
    290                 295                 300

Ser Asn Thr Tyr Ala Asp Arg Asp Asn Arg Asn Ala Cys Ser Tyr Asp
305                 310                 315                 320

Met Lys Val Ser Lys Thr Ser Met Val Ser Ala Asn Ser Met Gly Gly
                325                 330                 335

Thr Gly Thr Thr Val Arg Met Ala Ser Ala Tyr Thr Tyr Gly Ser Ser
            340                 345                 350

Val Thr Ser Thr Ala Tyr Val Gly Thr Met Asp Gly Gly Asp Ser Arg
        355                 360                 365

Val Met Thr Asn Cys Tyr Ala Thr Ser Ser Asn Ala Thr Asp Lys Tyr
    370                 375                 380

Asp Arg Cys Arg Ala Ala Asp Ser Thr Val Asn Gly Ser Gly Arg Ser
385                 390                 395                 400

Val Met Arg Ala Gly Asn Tyr Asp Val Tyr His Cys Val Tyr Cys Asp
                405                 410                 415

Thr Val Asn Lys Cys Arg Thr Cys Thr Arg Arg Ser Gly Ser Asp Thr
            420                 425                 430

Arg Val Asn Gly Thr Arg Lys Gly Gly Ala Ala Met Ser Arg Ala Ala
        435                 440                 445

Ser Ser Gly Val Trp Ser Ala Thr Thr Met Ser
    450                 455
```

<210> 42
<211> 34
<212> PRT
<213> RAT UROMODULIN

<400> 42
Gly Val Gln Ala Ser Val Ser Lys Ala Ala Ser Ser Asn Leu Gly Phe
1 5 10 15

Leu Ser Ile Trp Leu Leu Leu Phe Leu Ser Ala Thr Leu Thr Leu Met
20 25 30

Val His

<210> 43
<211> 34
<212> PRT
<213> MOUSE UROMODULIN

<400> 43
Gly Val Gln Ala Ser Val Ser Lys Ala Ala Ser Ser Asn Leu Arg Leu
1 5 10 15

Leu Ser Ile Trp Leu Leu Leu Phe Leu Ser Ala Thr Leu Ile Phe Met
20 25 30

Val Gln

<210> 44
<211> 33
<212> PRT
<213> HUMAN UROMODULIN

<400> 44
Gly Val Gln Ala Thr Val Ser Arg Ala Phe Ser Ser Leu Gly Leu Leu
1 5 10 15

Lys Val Trp Leu Pro Leu Leu Leu Ser Ala Thr Leu Thr Leu Thr Phe
20 25 30

Gln

<210> 45
<211> 34
<212> PRT
<213> BOVINE UROMODULIN

<400> 45
Gly Gly Gln Ala Ala Met Ser Arg Ala Ala Pro Ser Ser Leu Gly Leu
1 5 10 15

Leu Gln Val Trp Leu Pro Leu Leu Leu Ser Ala Thr Leu Thr Leu Met
20 25 30

Ser Pro

<210> 46
<211> 42
<212> PRT
<213> TORPEDO

<400> 46
Asn Gln Phe Leu Pro Lys Leu Leu Asn Ala Thr Ala Cys Asp Gly Glu
1 5 10 15

Leu Ser Ser Ser Gly Thr Ser Ser Ser Lys Gly Ile Ile Phe Tyr Val
20 25 30

Leu Phe Ser Ile Leu Tyr Leu Ile Phe Tyr
35 40

<210> 47
<211> 42
<212> PRT
<213> PLACENTA

<400> 47
Thr Ala Cys Asp Leu Ala Pro Pro Ala Gly Thr Thr Asp Ala Ala His
1 5 10 15

Pro Gly Arg Ser Val Val Pro Ala Leu Leu Pro Leu Leu Ala Gly Thr
20 25 30

Leu Leu Leu Leu Glu Thr Ala Thr Ala Pro
35 40

<210> 48
<211> 41
<212> PRT
<213> DECAY ACCELERATING FACTOR

<400> 48
His Glu Thr Thr Pro Asn Lys Gly Ser Gly Thr Thr Ser Gly Thr Thr
1 5 10 15

Arg Leu Leu Ser Gly His Thr Cys Phe Thr Leu Thr Gly Leu Leu Gly
20 25 30

Thr Leu Val Thr Met Gly Leu Leu Thr
35 40

<210> 49
<211> 35
<212> PRT
<213> T. BRUCEI

<400> 49
Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Glu Pro Gly Ala Ala Thr
1 5 10 15

Leu Lys Ser Val Ala Leu Pro Phe Ala Ile Ala Ala Ala Ala Leu Val
20 25 30

Ala Ala Phe
35

<210> 50
<211> 36
<212> PRT
<213> HAMSTER

<400> 50
Gln Lys Glu Ser Gln Ala Tyr Tyr Asp Gly Arg Arg Ser Ser Ala Val
1 5 10 15

Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe
20 25 30

Leu Met Val Gly
35

<210> 51
<211> 44
<212> PRT
<213> RAT

<400> 51
Lys Thr Ile Asn Val Ile Arg Asp Lys Leu Val Lys Cys Gly Gly Ile
1 5 10 15

Ser Leu Leu Val Gln Asn Thr Ser Trp Leu Leu Leu Leu Leu Leu Ser
20 25 30

Leu Ser Phe Leu Gln Ala Thr Asp Phe Ile Ser Leu
35 40

<210> 52
<211> 36
<212> PRT
<213> T. BRUCEI

<400> 52
Glu Ser Asn Cys Lys Trp Glu Asn Asn Ala Cys Lys Asp Ser Ser Ile
1 5 10 15

Leu Val Thr Lys Lys Phe Ala Leu Thr Val Val Ser Ala Ala Phe Val
20 25 30

Ala Leu Leu Phe
35

<210> 53
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:SYNTHETIC

<400> 53
gaagggcccc caagagatcc aagtctcct 29

<210> 54
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:SYNTHETIC

<400> 54